STUDIES OF THE TOXICITY OF NICOTINE IN COMBINATION WITH VARIOUS ADJUVANTS.

by

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INTRODUCTION

1. General

Any material used in combination with nicotine to produce a more effective kill than would be otherwise obtained by using nicotine alone, may be termed an adjuvant of the nicotine. The adjuvant may or may not have toxic qualities in itself, depending on such factors as the species of insect concerned and the meteorological conditions at the time of application. The effect which an adjuvant has upon nicotine may be due to either physical or chemical factors but more probably to a combination of both. Physically, the adjuvant may bring about more effective spreading and sticking, with greater ability to withstand changing climatic conditions. The adjuvant may also produce a smothering effect. Chemically, as an "activator", it may hasten the evolution of free nicotine by the reaction of the basic and acidic radicals of the soap and nicotine sulphate. An adjuvant may also act chemically by producing an affinity between itself and the surface of the solid, through chemical combination with contaminants on the surface or by dissolving protective coatings, such as wax.

The most common adjuvants recommended for use in nicotine sprays are the various soaps. As a rule the soaps used in spraying practices are the fatty acid soaps of the alkali metals. Other adjuvants used are the amine soaps, vegetable oil soaps, fish oil soaps, caseinates, blood albumen, glue and soap bark. Recently, manufacturing concerns, in the hope of utilizing their by-products, have interested themselves in spreaders and stickers with the result that such materials are now being marketed under various trade names.

Results of field plot tests have suggested in some instances that the commercial brands of nicotine sulphate may not produce consistent results from season to season and that the addition of adjuvants may not increase the toxicity against a particular insect species. Accurate comparison of nicotine soap sprays in the field, where climatic factors, insect populations and developments are variable from place to place and season to season, may only be accomplished after extensively replicated and extended experiments. The results of laboratory experiments may not be precisely applicable to field conditions, but a precise collation of the toxicity of various nicotine sprays under controlled conditions may provide valuable basic information for subsequent operations in the field.

The present work is concerned with experiments on the toxicity of various commercial nicotine sulphates used alone and in combination with some of the fatty acid soaps of the alkali metals, using <u>Drosophila melanogaster</u> Meig. as the test animal, with the ultimate object of collating the adjuvant value of the various soaps used. Certain aspects of the problem were suggested by Dr. W. H. Brittain, Dean of the Faculty of Agriculture, Macdonald College and Mr. Arthur Kelsall, who was formerly Officer-in-Charge, Dominion Entomological Laboratory, Annapolis Royal, N. S.

2. Problems Involved

Adults of Drosophila melanogaster were used as the test animal in all experiments. Results are therefore comparable with those of other workers engaged in similar research at Macdonald College where Drosophila flies are also being used as the test animal. The insect is ideal for investigations of this nature as it can be quickly reared in large numbers and is surprisingly resistant to nicotine.

As preliminary tests resulted in considerable mortality in check lots and results were often inconsistent, it was necessary to investigate the whole technique of rearing, handling, spraying, and feeding of flies to establish the factor or factors affecting the degree of susceptibility of the flies to nicotine. It also became evident that the technique would have to be adapted to handle conveniently larger numbers of flies than formerly for the following reasons: (1) The comparative testing of all solutions in the experiment as nearly as possible at the same time instead of the successive testing of the solutions on various dates results in a more accurate and simpler analysis of data. The experiment is limited, however, to the number of flies that can be conveniently handled in one day. (2) Replications should be made as quickly as possible, that is, if one testing of all solutions requires one full day, the following tests should be on following days until enough replications have been made to give consistent results.

The nicotines tested were limited to three commonly used brands of nicotine sulphates and one brand of the alkaloid. The nicotine sulphates tested were: "Neotine", manufactured by Chemicals Limited, Montreal, Quebec; "Hyco", manufactured by Hyatt Chemical Company, St.Catherines, Ontario;and "Britnico", manufactured by British Nicotine Company, Bootle, England. The alkaloid tested was "Nicofume", manufactured by Tobacco By-Products and Chemical Corporation, Louisville, Kentucky. Analyses of these brands of nicotines were made by Mr. F. A. Herman, Chemist, Division of Chemistry, Ottawa. Ten per cent solutions (by weight), were made up from the analyzed commercial brands and the spraying solutions made up from the ten per cent solutions by volume measurement.

As commercial insecticidal soaps often contain impurities in varying amounts, it was necessary to manufacture soaps in the laboratory for the work. The soaps were made by using amounts of the fats and alkalies indicated by the equations for their reactions.

In order to correlate results and physical properties, all spraying solutions were examined physically. This involved measurement of surface tension, "run off" points, and hydrogen ion concentration. All equipment had to be procured and set up, including an air pump and spraying apparatus to compare "run off" points, apparatus for nicotine analyses, tensiometer, potentiometer, controlled temperature and humidity cabinets, rearing jars and glassware.

3. Meaning of Toxicity

Wardle (83) states that no satisfactory definition for toxicity has been unanimously agreed upon by workers in the field. This can be readily understood when one realizes the scope of insecticidal research and the problems involved. Stomach poisons, contact poisons, and fumigants are all linked with toxicity, and what may be toxic or slightly toxic to one species of animal, may be non-toxic to another. The writer believes that toxicity is concerned with those properties of a material which produce greater or lesser lethal effects when administered to an animal.

4. Various Methods of Determining Toxicity

Investigators have used certain criteria in their comparisons of various insecticides for estimations of toxicity. Thus Marlatt (50) and Cook and McIndoo (15) compared various arsenicals by noting the time required to bring about death of certain larvae when fed definite amounts of these materials. Tartar and Wilson (75), Campbell and Filmer (12) and Lovett (44), quantatively analyzed the bodies of poisoned larvae for arsenic. Moore (56) in his trials used as criteria, the time required to kill, the amount of poison wash consumed and the ratio of the amount of poison found in the body to that amount found in the execreta. By taking the reciprocal of the number obtained by dividing the quantity of the chemical found in the execreta by that found in the body, the most toxic chemical becomes the one with the highest numerical value. Moore (55) and Peet and Grady (62) compared contact insecticides using the house fly, *Musca domestica*. Moore's results were based on the numbers of flies killed in 400 minutes, and Peet and Grady recorded the number "down" in 15 minutes.

Tattersfield and Morris (77) made tests with nicotines using *Aphis rumicis* as the test animal. The percentage affected in the test calculated to control is obtained by deducting the percentage affected in the control (a) from the percentage affected in test (b) and multiplying by $\frac{100}{100-a}$. The percentage affected calculated to control is then $\left(\frac{b-a}{100-a}\right)100$. The authors quote Mr.R.A. Fisher, Chief Statistician to the Rothamsted Experimental Station, which is given in part as follows: "The relation between concentration and probability of death could theoretically be determined by experiment by exposing a large number of insects to the action of insecticides at each concentration. The number of insects required, however, increases enormously if we wish in this manner to explore the region in which the probability of death is high. If as many as ninety-nine per cent of the insects were killed, the accuracy of the comparison between any two insecticides would depend upon the comparatively few insects which survived and to compare them with any accuracy, many thousands of insects would have to be used. The same difficulty arises in the comparatively unimportant cases where deaths are few. For a given number of insects the most accurate comparison can be made when the concentrations are such that about fifty per cent perish."

Marcovitch (48) based his comparisons of toxicities on the time required to kill fifty per cent of the larvae. The concentration was plotted against the reciprocal of the survival time (the velocity of fatality) and reveals a curve similar to that obtained by the action of lead nitrate on _Leuciscus phoxinum_ as worked out by Carpenter. This curve appears to correspond to the equation $K = \frac{1}{t} \log \frac{1}{\text{conc.}}$ where t = survival time and K a constant expressing a numerical value of toxicity. Campbell (10) describes a method of comparing toxicity by placing drops of the solution under test in the mouth of a feeding caterpillar. The amount imbibed is arrived at by weighing. The survival time is then determined by the relation between the dose per unit weight of insect and its effect. Instead of plotting doses against survival period, they were plotted against reciprocals of survival periods, thus representing speed of toxic action. If there is a direct relationship between dose and speed of toxic action it is represented by a straight line; if not, deviations from the straight line may be significant.

Trevan (79) comparing various drugs by injection into mice illustrates his results by a curve showing the percentage mortality obtained with each drug. The curve so obtained he called the "characteristics for the drug" and suggests that "the curve expressing the percentage of mortality, or some other limiting biological effect, produced by varying doses of a drug on animals of a certain species, shall be called the characteristic for that drug, effect, and species." He suggests the use of the medium lethal dosage as a criterion of toxicity, as the "certainly lethal dose", and the "maximum tolerated dose" have indefinitely large errors. Campbell (11) and Shepard and Richardson (72) considered the "knock out point" a good criterion, assuming that insects will die when so affected.

Bliss (3) interprets the sigmoid dosage mortality curve as a cumulative normal frequency distribution of the variation among the individuals of a population in their susceptibility to a toxic agent, which susceptibility is inversely proportional to the logarithm of the dose applied. In support of this interpretation is the fact that when dosage is inferred from the observed mortality on the assumption that susceptibility is distributed normally, such inferred dosages, in terms of units or probits, give straight lines when plotted against the logarithm of their corresponding observed dosages. This transformation to a straight regression line, facilitates the precise estimation of the dosage-mortality relationship. Bliss in a later paper (4), presents an extension of methods for computing the dosage-mortality curve as a straight line to cover some of the more frequent applications of the curve.

II. THE INFLUENCE OF PHYSICAL FACTORS OF SPRAY SOLUTIONS UPON TOXICITY.

A vast amount of literature has been written on the effects of wetting agents, spreaders, stickers, and activators, and their resultant effects upon toxicity. As yet no definite criterion for measuring toxicity has been established and the impossibility of such being established is clearly indicated when Hensill and Hoskins (37) say that chemical tests and measurements of physical properties, such as surface tension and angle of contact, are of little value in themselves. Before taking up the discussion of these physical characters, it is necessary to explain the terms "wetting" and "spreading".

1. Wetting and Spreading.

A simple laboratory test for the determination of the relative wetting powers of various solutions is in urgent demand, and various methods of comparison have been suggested from time to time. Cooper and Nuttall (16) state that results obtained have been of little value because the methods suggested were not based upon an exact knowledge of the principles underlying the process of wetting. Various definitions have been given for wetting and distinctions made between wetting and spreading. Hamilton (31) states that wetting occurs when the liquid comes in direct contact with the solid, the layer of air being excluded. Spreading occurs when the pull exerted by the solid to become wet is greater than the pull exerted by the surface of the liquid to air. Moore and Graham (58) state that a slight chemical affinity between liquid and solid is denoted as wetting or adhesion between the liquid and solid. If a liquid is brought into contact with a solid, wetting takes place; spreading may or may not occur.

Green (30) and Woodman (86) (87) state that wetting occurs when an object, on being dipped into a liquid, cannot be completely separated from the liquid by the simple process of emergence, for a film of the liquid adheres to the solid. Hensill and Hoskins (37) state that a wetting agent is any substance which increases the readiness with which a liquid makes real contact with a solid, i.e., wets it, if necessary by displacing a previous contaminant on the solid. A spreader is a material which increases the area that a given volume of liquid will cover on a solid or on another liquid.

2. Surface Tension

Burns (8) states that the cohesion or attractive force of molecules within a liquid gives rise to intrinsic pressure which cancels out except on the surface layer where a state of strain is established. As a result, surface molecules are arranged parallel to each other and at right angles to the surface, and therefore, have a larger number of molecules per unit area than the interior. The generally accepted test for determining the relative wetting powers of various solutions is the measuring of their surface tensions. Cooper and Nuttall (16) citing Brunswick and Smith, state that the wetting power of any liquid depends primarily upon its surface tension.

Vermorel and Dantony (81), distinguished between the "dynamic" surface tension of a new surface and the "static" surface tension of an old surface, claiming that the static surface tension affords a satisfactory criterion of the wetting power. Later, in 1912 (82), they say that the surface tension is not sufficient to determine the wetting power of a solution. Lefroy (43) points out that, as the tension air/solid remains constant and the tension wash/solid is intermediate, the tension wash/air is the only one to be considered, and the lower this tension, the more readily will the wash wet. O'Kane (59) (61) states that surface tension alone cannot be relied upon as an index of trachea penetration. A sodium oleate solution of 0.05% strength with a low surface tension gave only slight penetration to meal worm and cabbage worm trachea. An 0.5% solution of slightly higher surface tension exhibited marked penetration to both types of larvae. Woodman (86) (87) quoting Esser, states that diminishing the liquid/air tension is of the greatest importance and is apt to be overlooked, while undue stress is probably laid on the solid/liquid interfacial tension. O'Kane (59) further discusses surface tension, but this will be taken up in discussing adsorption phenomena.

3. The Angle of Contact

When a drop of liquid comes in contact with a solid its further activities, omitting certain complex factors, are dependent upon the behaviour of three forces, namely: the surface tension of the liquid, the apparent surface tension of the solid, and the interfacial tension of the liquid/solid. The surface tension of the liquid will tend to prevent its extension, the surface tension of the solid will effect the influence of the surface tension of the liquid, and the interfacial tension will reinforce the surface tension of the liquid. When all forces are in equilibrium the extension of the liquid ceases, and according to O'Kane (59) a definite angle of contact is reached between the liquid and solid. This may be expressed in the formula of E. L. Green (30): for equilibrium $T_2 = T_{1,2} - T_1 \cos\theta$ or $\cos\theta = \frac{T_2 - T_{1,2}}{T_1}$ when T_2 = surface tension of solid, $T_{1,2}$ = interfacial tension, and T_1 = surface tension of the liquid.

Green (30), quoting Quincke and others, assumed the angle of contact θ defined the tendency of a drop of liquid to spread over a solid, which tendency could then be measured by measuring the angle θ. The angle θ may not be less than zero and not more than 180 degrees. Over this range the wetting power is great for small angles and small for great angles.

English and Stellwaag according to Green (30), devised an apparatus to measure the angle of contact of a liquid on a solid as a means of defining the intensity of wetting. It includes a wide-mouthed container for the liquid, a means of holding, raising, immersing and turning the specimen of the solid, and a device for measuring the angle which the surface of the solid makes with the horizontal free surface of the liquid. Green (30), with similar apparatus, found that although there appeared to be an end point, a thin sheet of liquid could still be seen above the line of contact on the object, whether glass or twig. This is contrary to the behaviour required by the theory of end-point. They conclude that data depend more on the characteristics of the apparatus than of the solutions involved and results may be affected by contamination, selective absorption of solutions and atmospheric gases. Glass may not be used as a reference surface in comparing tree washes, because (1) the apparatus permits the operator to deceive himself into the belief that the angle of contact is much greater than it really is, unless the observation is confined to an ultimate film of liquid no wider than the range of action of the molecular forces and above which is perfect dryness; (2) no information regarding the ease of displacing the air film and other factors tending to retard spreading; (3) the materials to be wetted are not suitable to measurements of the angle of contact. Green (30), citing Ramsay and Shields, and Lord Rayleigh, found that zero indicated the angle of contact of water and glass.

Richards and Carver (66), and Green (30) quoting Bosanquet and Hartley, describe the search for a break in the narrow beam of light reflected from the region about the line of contact between water and glass. Had a real angle of contact occurred that was greater than thirty minutes of arc, they believe they would have found it. According to Woodman (86), it is necessary to reduce the contact angle to zero in order to produce wetting.

4. Interfacial Tension, Surface Concentration and Foaming Properties.

Cooper and Nuttall (16) quote the experiment of Plateau, stating that the shape of a drop of oil in dilute alcohol is the result of a tension exerted at the interface of the oil and dilute alcohol, and for the "sake of distinction is usually known as the interfacial tension". They further state that a drop of liquid will wet more effectively if the interfacial tension is reduced, even if the surface tension remains high, and conclude that the "interfacial tension rather than the surface tension is the determining factor in wetting power".

Other factors concerned with wetting are the solvent properties of the wetting liquid and surface concentration. The former needs no explanation. Increasing the concentration of an aqueous solution of a substance tends to aggregate the solute in the surface layers, resulting in a peculiar superficial viscosity or rigidity having high wetting power. The wetting power of such solutions seems to depend largely upon their capacity to form liquid planes, the high superficial viscosity of which prevents rupture and running together to form drops.

In discussing the foaming properties of a liquid in regard to wetting, Cooper and Nuttall (16) state that the generally accepted view of the nature of foam is that it is an emulsion of air in a liquid. It must possess a high surface viscosity of intervening film and an interfacial tension so low as to be incapable of breaking this film. The property of giving a lasting foam indicates that the liquid possesses the property of surface concentration and a low surface tension, such as have soap solutions. It is therefore clear that foaming power is in no way indicative of high wetting power.

In determining the wetting power of an animal dip or spray fluid, Cooper and Nuttall (16) used as a standard a thick paraffin oil surface, the surface tension of which was established. The wetting power of any two preparations can then be determined by finding their surface tensions and their interfacial tensions towards the standard oil. Cupples (17) (18) (19) (20) established a spreading coefficient for a number of soap solutions, using the same principle as Cooper and Nuttall. A drop of the solution to be tested is placed in a thin film of refined mineral oil and will spread only if it has a positive spreading coefficient. The surface tension of the oil is known and the surface and interfacial tensions are determined.

5. Relation of Adsorption to Toxicity.

O'Kane and co-workers (59) (60) (61), investigated surface activity with special reference to contact insecticides. They proposed that in a given series of related toxic compounds which exhibit surface activity, the relative toxicity is influenced by relative molecular concentration at interfaces and the resultant degree of surface activity. Surface activity is, therefore, conceived as affecting toxic action by bringing about increased concentrations of compounds at surfaces. It involves adsorption phenomena and is correlated to surface tension by the well-known equation of Gibbs. When certain data of Tattersfield is applied to Gibbs' equation it is shown that as the OH groups become greater, adsorption at the water/air interface becomes less, so correspondingly, concentration must be increased to maintain toxicity.

O'Kane et al (56), quoting Richardson, states that nicotine is most toxic at the higher pH values and under such circumstances the alkaloid is largely in the form of molecules of free nicotine, whereas in the case of nicotine of neutral or acid reaction, it is largely in the form of ions. Alterations in toxicity, brought about by a change in pH, may be due in part to alteration in surface activity. It is recognized that the change in toxicity may be due likewise, in part or even largely, to the ability of the undissociated molecules to pass through membranes.

O'Kane (59) sets forth on arithmetic probability paper, results of nicotine spraying experiments obtained by Tattersfield and Gimingham, which should approach a straight line but which do not, the curve resembling a typical adsorption curve. By replotting these data on logarithmic paper it was found that they approached a straight line. It is therefore inferred that nicotine solutions in successive concentrations do exhibit the phenomena of adsorption and that part of the toxicity exhibited by nicotine, as concentration is increased, is related to surface activity and adsorption phenomena. Certain substances, however, because of their physical properties, do not show adsorption curves above certain concentrations. Sodium oleate is not a true solution in that it is partly colloidal and its physical properties do not remain constant on increasing its concentration.

That orientation of molecules at the surface of water is important in the adsorption by ash-free charcoal is pointed out by Milner (54). In certain isomeric benzene derivatives, the amount of the derivative adsorbed appeared to be influenced by the position of the various groups in the benzene ring. If molecular orientation plays a part in adsorption phenomena, as indicated by the above, it would be expected to show an influence upon toxicity. O'Kane (59) states that evidence of molecular orientation at surfaces is obtained through studies of surface tensions. If, in two related compounds, one has a higher surface tension than the other, it is assumed that some evidence is offered to show that the molecules of the compound having the higher surface tension are oriented in such a way that the surface contains groups which are more active or which are stronger in "electro-magnetic stray fields of force".

III. HISTORICAL OUTLINE.

1. Nicotine

Nicotine, the principal alkaloid of tobacco, derives its name from Nicotiana, a genus of solanaceous plants to which the tobacco plant belongs, so-called after Jean Nicot, who first introduced tobacco into France in 1560. The presence of a volatile, poisonous principle in tobacco was first recognized by Vauquelin in 1809. The isolation of nicotine was first accomplished by Posselt and Reimann in 1828. Its structure was established by Pinner (1891-1895) as the ditertiary base beta-pyridyl-alpha-N-methylpyrrolidine, CHN (7).

Nicotine is an oily, colorless liquid, almost odorless when pure, but developing a tobacco-like smell on standing, and rapidly turning brown when exposed to air. It is highly toxic to insects as well as to man and other animals, and this toxicity to insects makes it of economic importance (7).

The earliest known record of the use of tobacco as an insecticide is that of Jean de LaQuintinze, who in 1690 wrote that 'tigres' infesting peach trees were washed with tobacco water with no effect (41). In 1734 John Bartram, in giving directions for packing botanical materials, suggested the use of tobacco leaves as a protection against insect injury (53). This is likely the earliest reference in which tobacco was recommended as a repellant. In 1746 W. D. Waite and others advised to squirt by means of a hand engine, water in which tobacco leaves had been soaked, to combat plum curculio in nectarines (80).

Hollister (39) states that it is not known when tobacco was first used as an insecticide. He states further that it was recommended in France in 1763 as a remedy for plant lice and was used in water and dust form. He finds that it was first used in America by Yates at Albany in 1814 as a control for sucking insects, and that William Corbett, in the "English Gardener", 1829, recommended tobacco juice for wooly aphis. Hollister also found that Thomas Fessenden, in the "New American Gardener", 1832, included tobacco in a list of materials which, he stated "may annoy or completely destroy insects".

R. Weston (84) in 1773 writes of a newly invented fumigating bellows to destroy insects with tobacco smoke. An unknown author of "Riego E Insectos in Semanario de Agr. y Astes.", Madrid, 1800, states that vegetables infested with aphids were successfully sprayed with a pinch of tobacco snuff in a cupful of water (2). E. Darwin (21) writes that in 1800 tobacco as an insecticide was in common use, a powder puff being used to blow the dust; while a tent over a nut tree was first used in fumigating with tobacco smoke.

In 1814, A. Hay (34) mentions a control for the blue insect, probably the woolly aphid, consisting of the juice of 4.0 pounds of roll tobacco, 4.0 pounds of flowers of sulphur, and 40 Scots pints of soapsuds. The concoction was effective in ridding the roots of apple trees of insects. J. MacKray, in 1814, (46) mentions a control for various caterpillars consisting of a decoction made by boiling together ½ pound of tobacco, 1.0 pound of soft soap and 18 Scots pints of water. These last two references are the earliest the writer could find of the use of soap in combination with tobacco.

F. Sang in 1814 (70), mentions the use of a force pump in the application of tobacco liquor as a control for the leaf roller Phalaena asperona. In 1825 G. S. MacKenzie (45) mentions the use of tobacco smoke in green house control. J. Strauch (75), writing in 1877, states that aphids were killed on potted plants by placing the plants in a closed box and then putting tobacco juice from his long-stemmed pipe on a hot-plate within the box. In 1884 Van Hulle (80), gives a control for greenhouse insects by placing tobacco extract on the heating pipes or on hot metal plates.

Concentrated tobacco extracts in Europe date from 1882, when Girard (29) visited a greenhouse in Paris and saw thrips, scale insects, and flies readily killed by a cloud of steam saturated with nicotine. This was obtained by boiling tobacco juice (at 14o Baume) in a flat pan until it was all evaporated. In the same year Carriere and Andre (14) write that the following formula was used by Thierry: tobacco juice titrated at 10°C, 5 parts; ammonia, 1 part; and water 4 parts. After applying this mixture to orange trees with a brush, the trees should then be sprayed with plain water, whereby all insects are washed off.

In 1890 tobacco extracts were first used in America when the report of the Entomologist (45) states a complete control of the cabbage butterfly, Pieris rapae, was obtained by the use of X. O. Dust. This dust consisted of finely ground tobacco with an admixture of carbolic acid. Carpenter in 1931 (13), states that in 1892 the first standardized extract of nicotine called "Rose Leaf" was placed on the market. It contained less than three per cent actual nicotine and as early as 1898 had a wider range of application than any form of insecticide used up to that time. The same author states that in 1910 Arnold developed a 40 per cent solution of sulphate of nicotine which is available at the present time in the form of "Black Leaf 40". Felt (27) states that, "Since the appearance in 1885 of 'Gold Leaf Tobacco Extract', a forerunner of B. L. 40 and B. L. 50 of the present, nicotine has been of increasing importance as a contact insecticide".

The first standardized tobacco extract did not appear in Europe until 1908, when Schwartz (71) recommended the following as a control for aphids: 3 parts tobacco extract (titrated and guaranteed to contain 8-9 per cent nicotine), 6 parts soft soap, 5 parts denatured alcohol and 136 parts of water. Rabate (63) says that the state factories of France prepare nicotine, ordinary and titrated tobacco juices for the agriculturists. The ordinary juice is obtained by leaching waste material in the tobacco factories. The titrated juice is distilled from ordinary juice and during the process sulphuric acid and sodium carbonate are added. The final product contains sulphate of nicotine, and small quantities of sulphate of sodium and carbonate of sodium. The mixture has a nicotine content of 10 per cent.

2. Nicotine Adjuvants.

The amount of literature describing experiments and recommendations made for insect control involving nicotines and soaps is voluminous. It is apparent that soaps were used as a means of controlling insects shortly after it was discovered that tobacco had insecticidal properties. The very thorough bibliography by McIndoo, Roark and Busbey (52) on the insecticidal uses of nicotine and tobacco, published in 1936, contains some 2497 important abstracts. In this publication it is shown that in 1814 Hay (34) recommends tobacco, sulphur and soapsuds as a control for the blue insect, probably the woolly aphid. In the same year, MacKray (46) recommends tobacco and soft soap as a control for the gooseberry caterpillar. From 1814 to the end of the century references to the use of soaps in nicotine sprays are not infrequent. By 1914 the use of soaps in nicotine became a common practice and in the following years a great deal of scientific investigation has established the status of many types of soaps and other materials as adjuvants to nicotine sprays and dusts.

Smith (73) states that above certain concentrations soap causes a loss of both spreading and wetting which is due to a chemical change affecting the physical properties of the nicotine solutions but not the nicotine. Ruggles (69) writes on the new insecticide, nicotine oleate, and states that it is more efficient if oil is emulsified with it. In the same year (42) Lees gives a formula for a nicotine-paraffin emulsion, stating that perfect wetting is necessary before a complete control is obtained. Wilson, DeOng, Worthley and Driggers, (85) (22) (88) (23), published separate papers comparing the toxicity of soaps in nicotine solutions. Headlee (36) writes on the comparative toxicities of pyrethrum and nicotine sprays, claiming that the addition of sodium oleate will reduce surface tension, permitting penetration of solution into breathing pores.

Hoerner (38), writes on the testing of sulphonated oxidation products of petroleum for toxicity and states that 0.5% Penetrol in nicotine 1-4000 is effective against aphids. McDonnell and Graham (51) find that, depending on the type of soap, soap-nicotine preparations on the market decrease in nicotine content during storage, if not protected from the air. Headlee (36) in working on a control for codling moth reports that nicotine tannate is very effective, but concludes that a great deal more research is to be done on it before recommending it for practical use. Hartzell (32), in working out a control for the pear psylla, reports that with the use of Penetrol a lower free nicotine content may be used and further states that nicotine in amounts above the maximum does not result in as great a control as when other compatible ingredients such as Bordeaux are also added. He concludes that when such ingredients of specific toxicity are mixed together they should not be termed "activators", but better say that they act by the principle of "summation". Hoerner (38) and Filmer (28) conclude that Penetrol in combination with nicotine is more effective than the fish oil soaps. Felt and Bromley (27) conclude that, with nicotine activators, results are dependent on the following factors: (1) stage of insect, (2) type of plant, (3) spreading and wetting qualities, (4) rapidity of evolution of nicotine, (5) climatic factors.

Shepard and Richardson (72), compared nicotine base and nicotine sulphate against <u>Aphis rumicis</u> by the immersion method. Curves constructed from data collected show the greatest variation at each end of the curve, although the standard deviation is greatest near the middle of the curve, which is due to the maximum "spread" or deviation in the individual measurements. Richardson (67) reports that, with 0.25 per cent potassium oleate, rotenone is more toxic than the pyrethrins or nicotine against the red spider mite. Huckett (40) states that the best control obtained for aphids is Penetrol and nicotine. Moore (57) published a new development in the fixation of nicotine. Nicotine, resorcinol and formaldehyde were heated together and the resultant precipitate was found to be 22 per cent nicotine, which is about one-fifth as soluble as the nicotine in nicotine tannate, and therefore its effect was more lasting than nicotine tannate. Driggers (23) obtained similar results and also found that Black Leaf 155 was the least effective of the nicotine compounds.

Eddy (24) gives his formula for a new spreader called Spreader 385 or Taroleate spreader which, when used in one part to 1000 of nicotine and water solution, spreads as well as four parts of liquid soap spreader in 1000 parts of nicotine and solution.

Tate and Andre (76) made laboratory toxicity tests of nicotine and sodium oleate solutions against onion and gladiolus thrips, and found that a fifty per cent mortality was obtained with a three per cent solution of nicotine and soap with gladiolus thrips. With the onion thrips, a 0.3 per cent strength of the same solution gave the same kill. Richardson et al, (68) compared nicotine, nornicotines, and anabasine upon *Aphis rumicis*,and concluded that anabasine was the most toxic nor nicotine somewhat less so. Ritcher and Colfee (64) (65) report that free nicotine may be directly incorporated in highly refined petroleum oil base to give a stable solution containing one to three per cent nicotine, and that it does not burn tender foliage. In the laboratory, nicotine and oil gave a high kill to such insects as mealy bugs and white fly. Eddy and Sharpe (24) and Eddy and Meadows (25), find that Karaya gum increases the efficiency of nicotine sprays. Using the gum 1:500, one-fifth to one-third of nicotine sulphate was effective.

IV. THE DEVELOPMENT OF TECHNIQUE FOR THE PRODUCTION OF DROSOPHILA MELANOGASTER.

1. The Adoption of Drosophila Flies as the Test Animal.

In the courses of preliminary work on insect toxicology at Macdonald College, H. A. Gilbert, J. Marshall, M. Prebble and others (in MS.) made tests of nicotine sprays against *Myzus persicae* and *Drosophila ampelophila* using the apparatus described by Tattersfield and Morris (77). The writer also used *Drosophila* in a few preliminary tests at Macdonald College and because of this and the fact that his results would be comparable with those of other workers at present engaged in similar experiments at Macdonald College, *Drosophila* were used in this project. The flies are easily reared in large numbers in the laboratory at any time during the year and their resistance to contact sprays makes them an ideal insect to work with. As *Drosophila melanogaster* were easily obtained, this species was used in all experiments. Culture tests with *Drosophila hydei* were discarded as the life cycle of this species is of somewhat longer duration than that of *D. melanogaster*.

2. Culture Tests.

Drosophila cultures were originally made up at Macdonald College by cutting bananas in small pieces and soaking in water to which a few teaspoonfuls of Brewer's stock yeast had been added. After being well shaken, the excess water was drained off and the mixture put into pint-size milk bottles which were used as rearing jars.

As a great many bottles, involving considerable handling would be required in rearing large numbers of flies and as the bananas would run to a considerable expense, rather extensive attempts were made to rear <u>Drosophila</u> on other materials, in gum or candy jars.

The synthetic solution devised by Raymond Pearl was first tried, and though it might be suitable for rearing flies for genetical studies, the rate of reproduction was too slow for the present needs. Attempts to rear <u>Drosophila hydei</u> and <u>Drosophila melanogaster</u> were made with cornmeal and National Breweries yeast and this medium proved ideal for larval development if the larvae became established before molds developed; otherwise, larvae would not develop. Mixtures of prunes, cornmeal, and yeast produced few flies although little mold occurred. Grapes, boiled apples and bananas were tried in combination with cornmeal and yeast but were discarded because of molds. Haydak (33) writes that glycerine in media for stored grain pests inhibits the growth of molds. Glycerine in varying quantities was mixed with cornmeal and yeast but at the point where molds disappeared, all flies died. Heating the cornmeal before adding the yeast and flies did not overcome the difficulty. On boiled or raw raisins, little or no mold occurred but the flies did not reproduce at all rapidly. Alfalfa meal and wheat flour proved unsatisfactory. Boiled apples mixed with fine sawdust and yeast gave promising results but emerging flies died within a few days unless fed on more favorable food, such as banana juice. At this point the attempt to rear D. hydei on materials other than bananas and yeast was discontinued.

The formula of Helen Redfield, mentioned in a paper by Bridges and Darby (6), was tried but proved unsuccessful. Larvae developed very slowly and the culture ingredients became too stiff for larvae to work through. The banana agar medium of Bridges and Darby was tried but exhibited no advantages over bananas alone. The alcohol banana medium of these writers was not tried but attempts were made to rear flies on cornmeal and three per cent by weight of alcohol. Although some good cultures were obtained a considerable number soon developed molds and the method was discontinued. Baumberger (1), quoting Lafar states, "From the standpoint of the oecological theory of fermentation, the alcohol produced by yeast should be regarded as a weapon capable of hindering the appearance of the fungoid competitors in saccharine nutrient media. However, when accumulated in the medium during the progress of fermentation it also restricts the further development of the producer". Bridges and Darby (6) state that Richards found the inhibiting effect of alcohol on the growth of yeast and fermentation to be very great. But on the usual bacteria-contaminated yeast cultures used as food for flies, the alcohol does not accumulate but is changed over by bacterial action into acetic acid. Bridges and Darby (6) show that the optimum hydrogen ion concentration for the growth of yeast is approximately 4.45. They also state that the exhaustion of the sugars and yeast food from the too little or too poor medium, is perhaps the primary factor in limiting the growth of flies.

Baumberger (1) found that Drosophila larvae died after twenty-eight days on sterile bananas, while those fed on yeast and bananas completed their larval period in five days. He found that the rate of growth was proportional to the amount of yeast used, twenty-four per cent yeast resulting in the greatest size, and that with lower percentages the larval period was lengthened while the ultimate size of the larvae was reduced.

3. Adopted Culture Medium.

Results of these tests indicated that a banana medium with yeast would be the most suitable and all cultures were therefore made up in the following way: Approximately one-half pint of fine dry sawdust is put in a gum jar and three or four bananas, depending on size, are partially peeled and dropped in whole. Approximately two dessertspoonfuls of National Breweries Yeast is shaken in upon the bananas and the culture is ready for the flies. After fermentation is well under way a little water may be added if the sawdust seems too dry for larvae to work through.

The sawdust takes up excess moisture, thus preventing drowning of larvae, and the skins of the bananas provide a place for pupation. The partially peeled bananas are more effective than the unpeeled bananas, as fermentation gets under way more quickly and the peeled area of the bananas provides a ready supply of food for the breeding flies.

4.. Tests with Various Breeding Chambers.

As early experimentation indicated some variation in susceptibility of the flies from various gum jars to the toxic action of nicotine, attempts were made to permit emerging flies to collect in one chamber. Flies for spraying tests would then be drawn from this common lot. Individual collecting from gum jars would also be eliminated. To this end, four methods were tested. These are explained, and the apparatus described, in the following paragraphs.

Method No. 1. This apparatus consisted of an upper chamber (Diagram 1) having a glass front, with an opening at the narrowing end where the flies were collected in a gum jar having a bottom of tulle. Air was passed through the chamber by holding an electric fan to an opening in the other end and the flies were thus driven into the collecting jar. The emerging flies passed up from the darkened cultures in the lower chamber by means of cotton sleeves fastened about the holes in the bottom of the upper chamber. Wire cones prevented the flies from re-entering the cultures. The apparatus was discarded because all emerging flies would not enter the upper chamber.

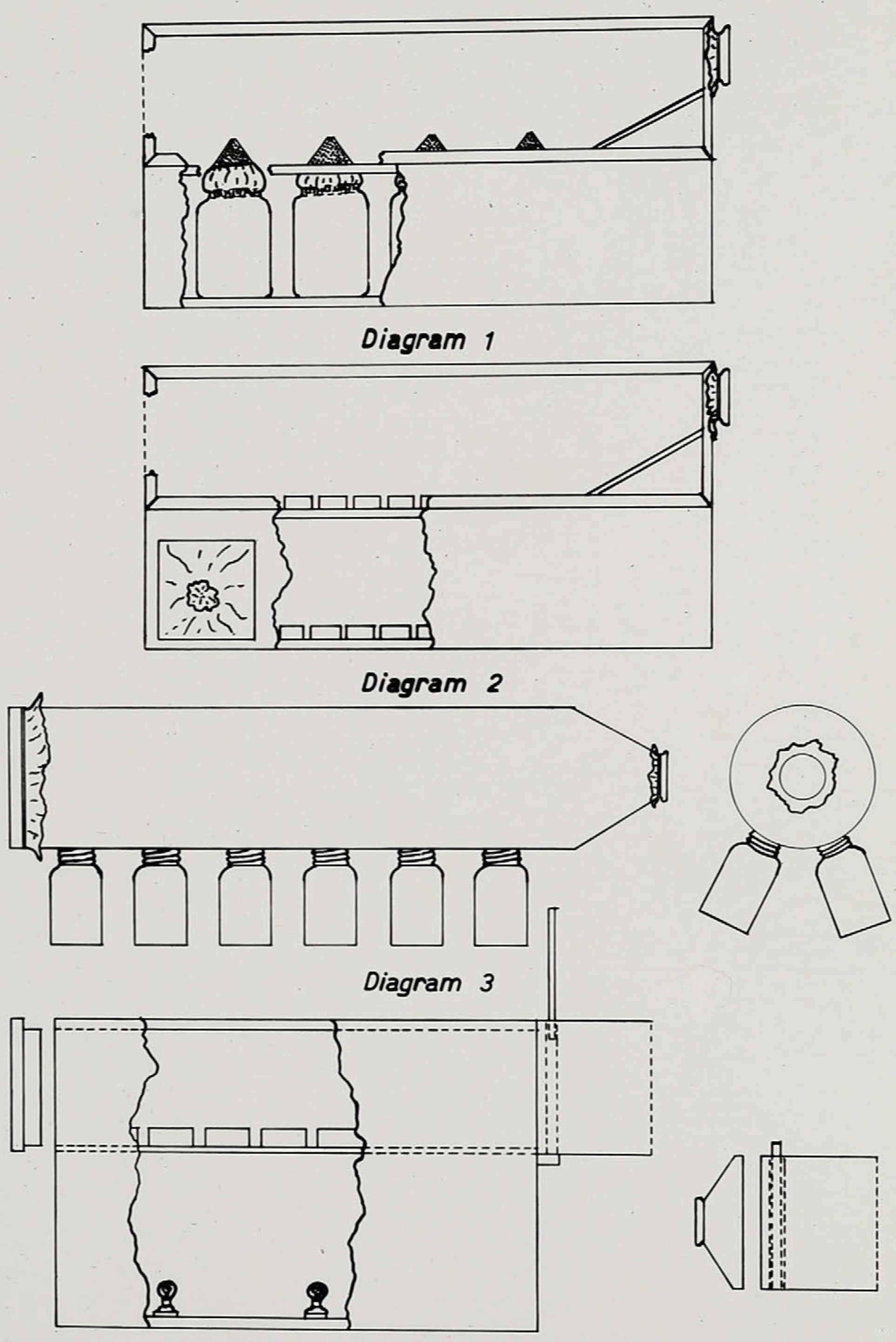
<u>Method No. 2</u>. Using the same chambers as in Method No. 1, the lower section was converted into a general breeding chamber, the culture medium being placed in galvanized flats 2 x 4 x 4 inches, (diagram 2). Before fly emergence began, the flats were transferred to the upper chamber through a sleeve fastened to the end of the lower chamber. The flies were collected as in Method 1. This proved more effective than the apparatus illustrated in diagram 1, but the emergence of flies per banana was lower than by the gum jar method.

<u>Method No. 3</u>. This apparatus consisted of a zinc cylinder 10 x 36 inches, to the lower side of which were soldered in two rows the tops of screw-top preserving bottles, (diagram 3). The culture medium was placed in the preserving bottles which were screwed into their tops on the collecting cylinder. The flies were driven into the collecting jar, held at the cone end of the cylinder, by a stream of air from an electric fan held to the tulle permanently fastened to the other end of the cylinder. Results were about the same as in the previous method. The sets of apparatus illustrated in diagrams 1, 2, and 3, were run at laboratory temperatures and humidities.

<u>Method No. 4</u>. A large controlled temperature and humidity cabinet was converted into a breeding chamber by fastening cloth over the door on the inside. Two zipper openings in the cloth provided a means for transferring cultures. These consisted of galvanized flats 10 x 4 x 2 inches. During pupation the

flats were transferred to an emergence box, (diagram 4). This was approximately 60 x 12 x 12 inches, of pine boards, with a zinc bottom through which heat penetrated from a chamber below, heated by electric bulbs. The thermoregulator was located in the upper chamber just over the cultures. Each day flats were made up and put in the breeding chamber, others were transferred to the emergence box, and still others were removed from the emergence box, made up with new medium and returned to the breeding chamber. Flies were collected from the emergence box as in former attempts, except that air was provided by a hand rotary duster. This apparatus was not a complete success as the flies would not all emerge in the emergence box. Temperature and humidity were approximately the same as in the gum jars and plenty of larvae were produced in the breeding chamber. At this point it was decided that, for further work, flies would be reared in individual gum jar cultures, the technique of which is described under the next heading.

EXPERIMENTAL BREEDING CHAMBERS



V. EXPERIMENTAL TECHNIQUE

1. Care of Cultures and Aging Jars.

During the period of time in which most of the spraying was done, it was found necessary to have on hand at least eighteen cultures to provide a minimum number of 5,000 flies per day. As the cultures depreciated very rapidly after fourteen days of emergence, it was necessary to discard and make up new cultures each day. They were made up as previously described, dated, and approximately 300 flies, of not more than three days age, were admitted to each culture. After an egg-laying period of four days, the flies were removed and discarded. Collections were made from the emerging cultures at the same time each day and were put aside in aging jars (Fig. 6) in a small temperature and humidity cabinet, from which, on the third day, they were removed for spraying. The temperature cabinet in which the cultures were kept was constructed of commercial wallboard and temperature was maintained at 80° F. The humidity inside the gum jars was usually about 95 per cent. Occasionally a culture had to be dampened with water. The drying was due to insufficient banana medium when made up, or to an excess of sawdust. Cabinets enclosed in glass were not satisfactory for ordinary gum jar cultures as condensation took place within the cultures and flies became stuck.

The aging jars consisted of gum jars the bottoms of which were replaced by tulle. Feeding was provided for by sewing a cotton cone to a hole in each cotton top and therein inserting a peeled banana. The cones hung down into the jars for four or five inches and excess juices from the cones passed through the tulle bottoms. It was noted that not more than 2,000 flies should be aged in one jar at a time. Cones had to be thoroughly washed out each time before using, otherwise they became stiff, preventing the passage of juices. The flies were aged at a temperature between 68 and 70 degrees Fahrenheit, and a humidity of 95 per cent. Lower humidities caused a partial drying of the cones, and prevented feeding.

2. Collecting of Flies.

In collecting emerging flies all cultures are removed from the temperature cabinet and placed in a row on the laboratory table. With a clean empty gum jar in one hand the cotton top of a culture is removed suddenly with the other hand and the gum jar placed over it upside down. Carried to the daylight the flies are drawn into the collecting gum jar, often being assisted by gentle shaking. When the culture is empty of flies, the collecting jar is suddenly removed and placed against the chest, preventing escape. The flies are easily jarred to the bottom of the collecting jar while held against the chest, and while they are there the cotton top of the second culture is suddenly removed and the collecting jar placed over it as before. With a little practice the culture and collecting jar can be brought together on a slant thus preventing flies already collected from spilling into the culture to be cleaned.

When the emergence for the day has been collected, the flies are transferred to the aging jars. Here a chemical separatory funnel is employed (Fig. 3). The rim of a gum jar metal cover is soldered to a small funnel and attached to the filling end of the separatory funnel with DeKhotinsky cement. The valve of the separatory funnel is closed and the rim is placed over the collecting jar, the cotton top of which has been removed. The two of them are then inverted and with a few downward sweeping motions the flies are transferred to the separatory funnel. A cork through which passes a piece of rubber tubing is then placed in the end of the separatory funnel. The valve end is then placed under the elastic holding the cotton top of the aging jar, with filled feeding cone already in place, the valve is opened and the flies tilted into the aging jars. The flies will clog in the valve end of the funnel if the flow is not regulated by slight suctions of air through the rubber tubing of the cork.

3. Admitting Flies to Spraying Tubes.

In preliminary investigations flies to be sprayed were put in tubes 45 mm. long and 14 mm. wide inside diameter, fifteen flies being admitted to each tube. This size was later discarded for a tube much larger, 60 mm. long and 35 mm. wide, inside diameter. Approximately 100 flies are put in each of these tubes. (If exactly one hundred flies could be admitted to each tube without too much trouble, it would save a great deal of time in recording spraying results and the final analysis could be carried out without the necessity of dealing with weighted averages and the conversion of percentages to "angles of equal information", as stated by Bliss (5). Flies for spraying are transferred from the aging jars to the separatory funnel in the same manner as previously described. Tulle, 28 mesh to the inch, is placed on one end of all tubes to be used. A small hand air pump is attached to the tubing leading through the filling end of the separatory funnel, which is placed on its side. The valve end of this is surrounded by a cork which fits into the open end of the spraying tubes and with a few gentle strokes of the air pump flies are driven into the spraying tube which is quickly removed and capped with tulle. Flies were not left in the separatory funnel any longer than necessary and all flies to be sprayed were not admitted to the separatory funnel at one time. When spraying large numbers of flies a second separatory funnel was used, the first being laid aside before evidence of moisture appeared. Flies were sprayed as soon as possible after being admitted to the spraying tubes and all spraying was carried out at exactly 70 degrees Fahrenheit, temperatures being recorded in the fume chamber at the level of the atomizer. The temperatures were controlled by electric fans placed near radiators, etc. It was impossible to control the slightly fluctuating humidity which approximated 40 per cent in the basement laboratory.

4. Spraying Apparatus

The apparatus consists essentially of an outfit supplying a current of air of an even pressure to an atomizer located in a fume chamber. A quarter horsepower motor, operating a small air pump of the intake valve type, supplies air to a pressure tank, 24 by 9 inches. The large drive wheel of an old type sewing machine, mounted on the shaft of the air pump, makes an ideal fly-wheel, and was found quite necessary for successful operation. A round leather belt from the belt groove of the fly-wheel to the motor operates the pump. Air enters the pressure tank through a car tire valve soldered to it. A turn-off valve is situated in the air line between the reducing valve and the pressure tank and is located near the atomizer. An air cleaner was found necessary and was made of heavy glass tubing containing an amount of absorbent cotton. It is located in the air line, just before the reducing valve. A manometer between the reducing valve and the atomizer records the exact air pressure in inches of mercury.

The atomizer used was Tattersfield's No. 2 type. The advantage of this atomizer over the No. 1 type is that it has an adjustment for regulating the fineness of the spray. Preliminary work with the former type of atomizer showed that one cubic centimetre of spraying solution of low viscosity would atomize in six or seven seconds, and with the No. 2 type the same solution and amount could be atomized in a period of time exceeding a minute if necessary, both being operated at a pressure of 15 pounds per square inch. With atomizer No. 2, and using the adjustment, solutions of varying viscosities can be atomized in the same period of time. The atomizer is located in a fume chamber and is mounted on a platform adjustable for height by means of nuts turning on four threaded metal legs. A spraying clamp, also adjustable for height, is located in such a position that the tube containing the flies is held directly beneath and with the upper end seven and one-half inches below the orifice of the atomizer. Its position is checked from time to time by means of a plumb bob. The angle of the spray cone is also frequently checked by spraying a piece of coloured blotting paper surrounding a spraying vial while in position.

5. Spraying Operations

The motor is started and when pressure reaches 25 pounds per square inch in the pressure tank, spraying is begun. Air is admitted to the atomizer by opening the shut-off valve from the tank and is regulated to a height of 15 inches of mercury by the reducing valve. In spraying, only one-half cubic centimetre of solution is used per tube, early experiments having shown this to be as effective as one cubic centimetre. In tests involving a few tubes the solution is admitted to the atomizer by a small pipette. Where more tubes per test are used, the solution is contained in a 25 cc. graduated pipette equipped with a valve and fastened in position by means of a laboratory stand. When the pressure reaches forty pounds in the tank the air pump is stopped and is not started again until the pressure has dropped to twenty-five pounds. Using an Eastman Timer, the atomizer is adjusted to atomize 0.5 cc. of spraying solution in 12 - 15 seconds. The tubes are sprayed as soon as possible, and in order in which they were made up. After having sprayed with one solution, distilled water is run through the atomizer, followed by a little of the next spray solution to be used in order to cleanse the atomizer of contamination before continuing spraying operations.

6. Feeding Flies After Spraying and Recording Results.

One hour after each tube was sprayed, the flies were transferred to corresponding feeding vials. Originally they were fed by forcing absorbent cotton plugs containing 10% dextrose solution into the neck of the vials. This was not satisfactory as some solution often escaped into the inside of the vial and caused the flies to stick to the glass. By putting absorbent cotton in small cones made of galvanized screening to fit the vials and then soaking in the feeding solution before inserting into the vials, escape of the solution was eliminated. It was also discovered that the dextrose solution dried quite rapidly, and in further experimental work eight per cent honey solution was used, as shown in feeding experiments to be described.

Feeding tubes were constructed of the same diameter as the spraying tubes, and somewhat longer. One end of the feeding tube was covered with tulle, the other end contained the feeding solution in absorbent cotton, held in position by cones of galvanized wire screening. The open feeding tube was more advantageous to use than was the vial, as most of the flies which became stuck to the sides of the tube during spraying could be dislodged by simply blowing down through the spraying tube into the feeding tube, thus eliminating considerable handling with camel's-hair brushes.

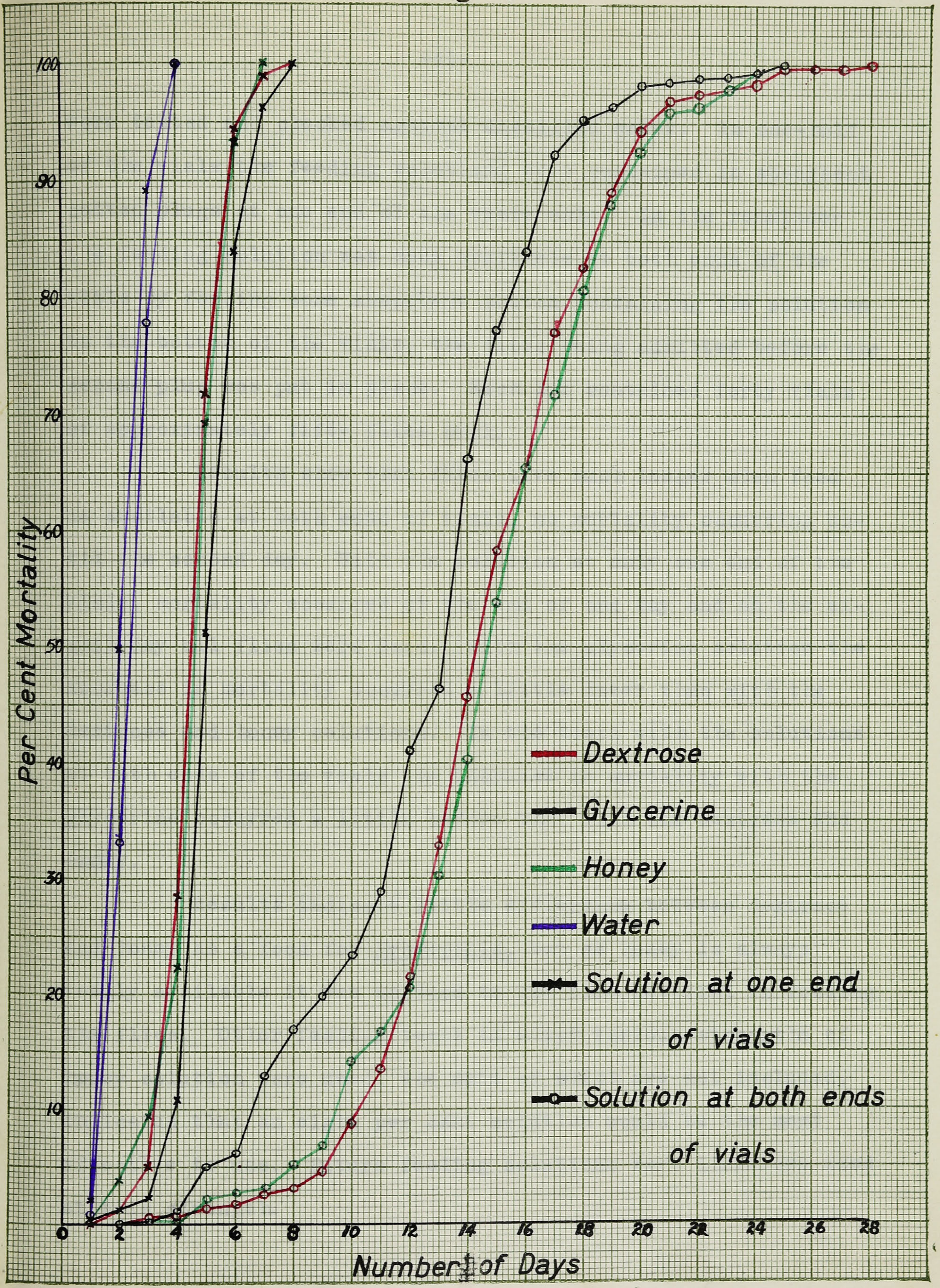
After feeding, the flies were removed to a temperature and humidity cabinet and held at 70 degrees Fahrenheit and at a humidity of approximately 95 per cent for twenty-four hours, when the dead and live flies were recorded. Originally the flies were held forty-eight hours after spraying, but results taken at 24 and 48 hours indicated that a lower co-efficient of variation occurred in the 24 hour recordings. In separating the dead from the living flies for counting, the live flies were attracted into a round bottom flask by light.

7. Feeding Tests.

The importance of providing proper feeding facilities for the flies after spraying was indicated in preliminary experiments. Feeding tests were therefore carried out, the results of which are shown in diagram 5.

Ten per cent solutions of dextrose, glycerine and honey

Diag. 5



Per Cent Mortality
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Number of Days
Dextrose
Glycerine
Honey
Water
Solution at one end of vials
Solution at both ends of vials

were tested and checked against distilled water. In one-half of the vials the feeding solutions were contained in absorbent cotton plugs at one end of the vials only. In the remainder the feeding solution was made available at both ends of the vials by forcing some absorbent cotton containing the solution to the bottom of the vials, the flies being admitted before the second plug was put in place. Each lot contained forty vials of approximately fifty flies each.

Results show that flies live much longer when food is available at both ends of vials, no mortality occurring until the second day. With the solution at only one end of the vials, mortality occurred at the end of one day and on the eighth day all flies were dead. With the glycerine solution the increase in mortality occurred a little later than with the dextrose and honey solutions. In the tests with the solutions at both ends of the vials the increase in mortality with the glycerine occurred a little before that with the dextrose and honey.

Results indicate little difference between dextrose and honey as a food for <u>Drosophila,</u> but as honey is always easily available it was decided to use this material in feeding the flies. Other feeding tests in which various strengths of honey solution were used, the data of which are not included, indicated that an eight per cent solution would be the most effective to use. Future feeding solutions were therefore used at this strength.

VI. PRELIMINARY SPRAYING TESTS

1. Median Lethal Dosage

Experiments to establish the median lethal dosage were run in preliminary work using the common brand of nicotine sulphate known as "Neotine". Flies were obtained from gum jar cultures producing a good emergence and the median lethal dosage for flies from this source was determined at approximately 0.5%.

2. Comparative Nicotine Tests

Using 0.5 per cent solutions for future comparisons, tests were made comparing three commercial brands of nicotine sulphate; Neotine, Hyco and Britnico, and the commercial alkaloid, Nicofume. Flies were secured from healthy cultures, the emergence from each culture being separated from other cultures and divided approximately equally between the four nicotines under test.

Results are shown in tables 1-8.

Table No. 1

Sprayed with 0.5% Neotine Solution.

Vial No.	1		2		3		4		5	
Culture No.	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill
1	73	33.3	105	47.6	53	30.5	122	48.3	106	17.9
2	121	51.2	119	49.4	91	48.4	107	56.1	112	41.8
3	74	89.2	78	77.1	81	96.3	98	95.0	72	94.5
4	63	42.8	167	41.7	138	54.3	162	41.8	134	44.1
5	143	68.6	77	66.2	114	43.0	154	66.2	133	48.2
6	167	67.1	165	70.3	111	43.2	131	58.2	73	43.8
7	100	41.0	129	43.4	118	53.6	201	54.6	---	---
8	111	53.2	126	56.3	179	65.3	26	61.5	98	35.3
9	151	54.7	91	44.0	95	41.0	106	57.6	---	---
10	91	51.7	88	40.9	114	46.5	116	47.4	57	56.2

TOTAL 5346

AVERAGE NUMBER OF FLIES PER VIAL 111.4

AVERAGE PER CENT MORTALITY 53.3%

Table No. 2

Sprayed with 0.5% Hyco Solution.

Vial No.	1		2		3		4		5	
Culture No.	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill
1	65	29.3	112	43.7	90	38.8	88	30.7	80	38.8
2	120	35.8	96	50.0	91	45.2	119	55.5	84	25.0
3	73	94.1	91	89.0	88	47.8	96	70.7	83	78.4
4	102	22.6	198	40.3	182	27.6	94	23.3	119	37.0
5	110	47.3	206	79.3	116	37.1	92	43.7	127	81.0
6	139	49.7	152	34.8	139	29.4	140	40.0	103	36.8
7	56	30.3	98	31.7	107	19.7	134	33.8	170	71.2
8	117	51.1	72	48.6	104	52.8	218	45.8	---	----
9	83	59.1	147	49.7	79	48.2	110	50.9	107	51.3
10	89	24.7	126	34.8	107	38.3	107	29.9	119	37.8

TOTAL 5545

AVERAGE NUMBER OF FLIES PER VIAL 113.2

AVERAGE PER CENT MORTALITY 45.3%

Table No. 3

Sprayed with 0.5% Britnico Solution.

Vial No.	1		2		3		4		5	
Culture No.	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill
1	111	21.6	119	34.4	106	27.4	141	31.9	106	33.9
2	99	33.3	73	56.1	112	35.7	95	30.5	104	42.3
3	95	84.2	80	80.0	42	92.8	121	83.4	97	84.5
4	154	62.2	116	39.6	128	39.0	97	42.2	63	39.7
5	142	71.1	145	82.7	166	70.5	129	87.6	183	53.0
6	136	56.6	137	62.0	121	47.9	132	46.1	153	40.5
7	89	35.9	146	40.4	154	45.4	126	36.5	136	28.6
8	114	58.7	106	41.5	138	38.4	115	40.0	148	56.1
9	73	69.8	114	56.1	104	43.2	117	59.5	---	----
10	98	42.8	115	46.1	119	42.8	101	48.5	---	----

TOTAL 5616

AVERAGE NUMBER OF FLIES PER VIAL 117.0

AVERAGE PER CENT MORTALITY 50.9%

Table No. 4

Sprayed with 0.5% Nicofume Solution.

Vial No.	1		2		3		4		5	
Culture No.	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill
1	51	37.2	88	55.6	59	66.1	59	33.9	113	34.5
2	74	43.2	153	62.1	109	79.2	121	88.4	77	32.4
3	94	98.9	128	100.0	82	100.0	89	98.8	90	98.8
4	71	91.5	113	71.7	219	73.5	83	89.1	143	91.6
5	110	92.7	156	83.3	102	84.3	98	85.7	143	89.5
6	137	89.0	131	76.3	123	94.3	121	81.0	132	86.3
7	161	74.5	190	88.4	155	87.7	40	97.5	144	87.3
8	87	80.5	118	96.6	90	86.6	153	90.2	122	87.7
9	134	86.5	146	99.3	156	85.2	144	93.7	118	82.1
10	114	81.6	109	76.1	102	80.4	127	95.2	136	84.5

TOTAL 5815

AVERAGE NUMBER OF FLIES PER VIAL 116.3

AVERAGE PER CENT MORTALITY 82.5%

Upon the suggestion of Dr. John Stanley, Queen's University, the results of the experiment were analysed as follows:-

Let x = number of flies in a given vial.

" y = number of flies killed in a given vial.

" p = per cent of flies killed in a given vial.

" Nv = the number of vials taken from one jar.

" Nj = the number of jars used.

Then $\overline{P}$, the mean per cent killed for the vials from one given jar, is:-

$$\overline{P} = \frac{S(y/x)}{Nv} = \frac{S(P)}{Nv}$$

For jar No. 1, Table No. 1 this would be:-

$$S(P) = 33.3 + 47.6 + 30.2 + 48.3 + 17.9 = 177.3$$

$$Nv = 5$$

$$\overline{P} = \frac{177.3}{5} = 35.46\%$$

The Standard Deviation σp for a given jar is:-

$$\sigma p = \sqrt{\frac{S(P^2)}{Nv} - \overline{P}^2}$$

For jar No. 1 this would be:-

$$\sigma p = \sqrt{\frac{33.3^2 + 47.6^2 + 30.2^2 + 48.3^2 + 17.9^2}{5} + 35.46^2}$$

$$\sigma p = \sqrt{130.6}$$

$$\sigma p = 11.45$$

Then for jar No. 1, Table No. 1, P

$$\overline{P} = \frac{35.46}{11.45}\sigma p = 3.1\sigma$$

As the level of significance is 1.64σ, $\overline{P}$ for jar No. 1 is significant.

The coefficient of variability for a given jar is:-

$$C.\ V. = \frac{100\sigma p}{P} = \frac{100(11.45)}{35.46} = 32.25\%$$

As the level for unsatisfactorily variability is 61%, jar No.1 <u>has satisfactorily low variability</u>.

The Standard Error of the Mean for a given jar is:-

$$S.E._{\bar{P}} = \frac{\sigma}{\sqrt{Nv}}$$

For jar No.1 this would be:-

$$S.E._{\bar{P}} = \frac{11.45}{\sqrt{5}} = \frac{11.45}{2.236} = 5.12 \text{ (approximately)}$$

A given jar differs significantly from the grand mean $\bar{P}$ if:-

$$\bar{p}-\bar{P} > 2.5\sqrt{(S.E._{\bar{p}})^2 + (S.E._{\bar{P}})^2}$$

Results of these analyses for Neotine are shown in Table 5.

Table No. 5

Showing results when sprayed with 0.5% Neotine Solution.

Jar No.	Mean % Mortality	Standard Deviation	Coefficient of Variation	$\frac{\overline{P}}{\sigma p}$	Standard Error of Mean	Significant Difference if $\overline{p} - \overline{P} > 2.5\sqrt{(S.E.\overline{p})^2 + (S.E.\overline{P})^2}$
1	35.46	11.45	32.29	3.1	5.12	$18.34 > 11.50$
2	49.36	4.83	9.78	10.2	2.16	$3.44 < 7.75$
3	90.42	7.08	7.83	12.77	3.16	$36.62 > 9.50$
4	44.94	4.75	10.56	9.46	2.12	$8.86 > 7.67$
5	58.44	10.65	18.22	5.48	4.76	$4.64 < 13.12$
6	56.52	11.34	20.06	4.98	5.07	$2.72 < 13.82$
7	47.91	5.80	12.10	8.25	2.90	$5.9 < 9.12$
8	54.4	10.23	18.80	5.31	4.57	$0.6 < 12.67$
9	47.1	16.28	13.33	7.50	3.14	$6.7 < 9.62$
10	48.54	5.14	10.58	9.44	2.29	$5.26 < 7.97$

GRAND STANDARD DEVIATION FOR ALL VIALS 15.46

AVERAGE PER CENT MORTALITY ($\overline{P}$) 53.3%

COEFFICIENT OF VARIATION 28.43%

GRAND STANDARD ERROR 2.2315

$\overline{P} = 53.3 > 1.64\,\sigma$ or 25.35. That is, $\overline{P}$ is significant.

Table No. 6

Showing results when sprayed with 0.5% Britnico Solution.

Jar No.	Mean % Mortality	Standard Deviation	Coefficient of Variation	$\frac{\bar{P}}{\sigma p}$	Standard Error of Mean	Significant Difference if $\bar{p} - \bar{P} > 2.5\sqrt{(S.E._{\bar{p}})^2 + (S.E._{\bar{P}})^2}$
1	29.80	5.04	16.91	5.91	2.25	$21.09 > 8.57$
2	39.58	9.13	23.06	4.33	4.08	$11.31 < 12.00$
3	84.98	4.22	49.65	20.14	1.89	$34.09 > 8.02$
4	44.54	8.89	19.96	5.01	3.98	$6.35 < 11.87$
5	72.98	11.97	16.40	6.09	5.35	$22.09 > 14.85$
6	50.62	7.68	15.17	6.60	3.43	$0.27 < 10.75$
7	37.36	5.54	14.83	6.74	2.47	$13.53 > 8.95$
8	46.94	8.63	13.38	5.44	3.86	$3.95 < 11.62$
9	57.36	8.14	14.19	7.05	4.07	$6.47 < 12.10$
10	45.05	2.40	5.33	18.77	1.20	$5.84 < 7.15$

GRAND STANDARD DEVIATION FOR ALL VIALS 18.02

AVERAGE PER CENT MORTALITY ($\bar{P}$) 50.92

COEFFICIENT OF VARIATION 35.41

GRAND STANDARD ERROR 2.57 $\bar{P} = 50.92 > 1.64\sigma$ or 29.55. That is, $\bar{P}$ is significant.

Table No. 7

Showing results when sprayed with 0.5% Hyco Solution.

Jar No.	Mean % Mortality	Standard Deviation	Coefficient of Variation	$\frac{P}{\sigma p}$	Standard Error of Mean	Significant Difference if $\bar{p}-\bar{P} > 2.5\sqrt{(S.E._{\bar{p}})^2 + (S.E._{\bar{P}})^2}$		
1	36.26	5.43	14.98	6.67	2.42	8.72	$<$	18.62
2	42.30	10.80	25.54	3.91	4.83	2.68	$<$	13.55
3	76.00	16.28	21.43	4.67	7.28	31.02	$>$	19.20
4	30.16	7.21	23.91	4.18	3.22	14.82	$>$	10.13
5	57.68	18.64	32.32	3.09	8.33	12.70	$<$	21.70
6	38.14	6.72	17.62	5.68	3.01	6.84	$<$	9.68
7	37.34	17.61	47.16	2.12	7.88	7.64	$<$	20.63
8	49.57	4.18	8.43	11.86	2.09	4.59	$<$	8.08
9	50.24	1.24	2.46	40.51	0.55	5.26	$<$	6.30
10	33.10	5.05	15.25	6.56	2.26	11.88	$>$	8.35

GRAND STANDARD DEVIATION FOR ALL VIALS 17.27

AVERAGE PER CENT MORTALITY ($\bar{P}$) 45.08%

COEFFICIENT OF VARIATION 38.33%

GRAND STANDARD ERROR 2.49 $\bar{P}$ = 45.08 $>$ 1.64 σ or 28.32. That is, $\bar{P}$ is significant

Table No. 8

Showing results when sprayed with 0.5% Nicofume Solution.

Jar No.	Mean % Mortality	Standard Deviation	Coefficient of Variation	$\frac{P}{\sigma p}$	Standard Error of Mean	Significant Difference if $\bar{p}-\bar{P} > 2.5\sqrt{(S.E._{\bar{p}})^2 + (S.E._{\bar{P}})^2}$		
1	45.46	13.42	29.52	3.38	6.00	35.55	$>$	16.25
2	61.06	21.29	34.87	2.87	9.52	19.96	$<$	24.62
3	99.30	0.57	0.57	177.53	0.25	18.29	$>$	6.25
4	83.48	8.95	10.72	9.44	4.00	2.47	$<$	11.77
5	87.10	3.50	4.02	24.03	1.56	6.09	$<$	7.64
6	85.38	6.25	7.32	13.66	2.79	4.37	$<$	9.35
7	87.08	7.33	8.42	11.88	3.27	6.07	$<$	10.27
8	88.32	5.22	5.92	16.92	2.33	7.31	$<$	8.52
9	89.36	6.26	7.01	14.27	2.80	8.35	$<$	23.20
10	83.56	6.41	7.68	13.03	2.86	2.55	$<$	9.47

GRAND STANDARD DEVIATION FOR ALL VIALS 17.69

AVERAGE PER CENT MORTALITY ($\bar{P}$) 81.01%

COEFFICIENT OF VARIATION 21.83%

GRAND STANDARD ERROR 2.50 $\bar{P} = 81.01 > 1.64\sigma$ or 29.01. That is, $\bar{P}$ is significant

Since a given gum jar culture differs significantly from the grand mean $\bar{P}$ if $\bar{p} - \bar{P} > 2.5 \sqrt{(S.E._{\bar{p}})^2 + (S.E._{\bar{P}})^2}$, replications show inconsistency between samples of flies from various cultures sprayed with any one brand of nicotine. Also replicate sprayings of each of the four brands of nicotine do not show great consistency when using samples of flies taken from the same culture, for each replication.

Conditions of rearing, handling, spraying, and feeding of flies in all gum jar cultures were the same so that the inconsistency of results must have been due to varying conditions of the flies. Tests were therefore run to establish the factor or factors causing the variation in susceptibility of the flies to the nicotine solutions compared. The following experiments established the effects of (1) the age factor, (2) the humidity factor, and (3) the culture age factor upon the degree of toxicity.

3. AGE FACTOR

Results from the tests of the four brands of nicotine sulphate using flies from average gum jars indicate inconsistency in results even when fairly large numbers of flies were used. To establish any possible effects of the age factor upon results it was decided to spray flies one, two, three and four days old with 0.5 per cent "Neotine nicotine sulphate. Flies were secured as described under method 4 and were aged by methods previously mentioned.

Results were as follows:-

Table No. 9

Age Factor.

Flies were sprayed with 0.5 per cent Neotine solutions.

Age	One day		Two days		Three days		Four days	
Repl.	No.flies Sprayed	% Kill	No.flies Sprayed	% Kill	No.flies Sprayed	% Kill	No.flies Sprayed	% Kill
1	403	54.8	502	10.3	60	26.6	481	9.9
2	124	69.3	152	25.3	213	22.5	238	18.1
3	699	27.7	564	8.8	560	9.6	148	6.7
4	888	44.3	871	10.4	857	11.4	975	11.3
5	284	40.8	845	8.0	755	13.9	731	16.7
6	306	44.4	316	27.2	726	17.2	663	16.8
7	666	44.3	255	11.3	162	24.6	522	18.7
8	517	43.8	---	----	682	13.2	367	7.9
9	247	36.0	343	13.9	378	19.8	343	19.5
TOTAL	4134		3868		4393		4468	
AVERAGE % KILL		42.5		11.9		14.8		14.3

GRAND TOTAL 16,863

Results in Table 1 show that the flies one day old are the most susceptible to nicotine, flies two days old, the least susceptible and flies three and four days old slightly more susceptible to nicotine than flies two days old. At this point it was seen that the apparatus in which the flies were reared (diag. 4) would not be suitable for extensive operations and it was therefore decided to continue with the gum jar method of rearing. As future results would be obtained from flies reared in gum jars it was necessary to carry on the experiment establishing the effects of age on toxicity using flies reared under the gum jar method. Results were as follows:-

Table No. 9a

Age Factor

Flies were sprayed with 0.5 per cent Neotine solutions.

Age Repl.	No.flies Sprayed	% Kill	No.flies Sprayed	% Kill	No.flies Sprayed	% Kill	No.flies Sprayed	% Kill
1	965	33.5	841	4.3	943	3.0	809	9.0
2	940	13.7	1264	3.0	1205	4.8	917	5.9
3	1076	31.4	1318	2.5	1147	2.7	1441	4.9
4	1628	12.5	1501	4.4	1442	1.8	1558	5.0
5	1387	15.6	1236	2.5	1376	3.5	1598	4.2
6	1568	16.5	1151	5.5	1457	3.5	574	5.2
7	1451	15.1	1241	2.4	1068	1.5	990	5.0
8	1562	11.1	716	2.9	1138	5.2	982	5.3
9	973	13.2	977	3.2	1314	7.6	1168	7.3
10	762	14.6	1271	3.2	1168	4.3	1650	7.4
TOTAL	12312		11516		12258		11687	
AVERAGE % KILL		17.1		3.4		3.8		5.9
GRAND TOTAL		47,773						

The average number of flies dead in Tables 9 and 9a was calculated from the total number of flies sprayed in each case. Results of Table 9a are consistent with those of Table 9 with the exception of the higher kill obtained in the four day old flies of Table 9a. It was found unnecessary to analyze the results as a daily per cent kill shows consistency and it is definitely shown that the age factor may in some cases, as, for example, flies one day old, affect the degree of toxicity. The higher mortality in Table 9 cannot be explained. It has been previously mentioned that the technique and method of rearing flies obtained from this source (diagram 4) had to be discarded because of the low emergence of flies per pound of bananas in comparison with the emergence obtained under the gum jar method of rearing. The lowest per cent kill obtained in both tables was with flies that were two days old and the per cent mortality obtained with the three day old flies was only slightly higher. All future tests were made, however, with flies three days old as other workers were using flies of this age.

4. Humidity Factor

To establish the effects of humidity upon toxicity it was decided to age the flies in three widely ranging humidities before spraying. Cylindrical cages were constructed of fine wire netting having a cotton bottom and a cotton top from which was suspended the cotton feeding cone containing the banana, being identical with the method employed in feeding in aging jars. The emergence of each day was divided into three lots and put into these cages and at the age of three days the flies were sprayed with 0.5 per cent "Neotine" nicotine sulphate.

The low humidity cages were put in a cabinet on the second floor of the laboratory, held at 70 degrees Fahrenheit and at room humidity which was approximately 40 per cent. Other cages were put in controlled temperature and humidity cabinets and were held at 70 degrees Fahrenheit and at a humidity of approximately 75 per cent. In the third group the cages were put inside gum jars containing moist sawdust and were held at 70 degrees Fahrenheit with humidity at approximately 100 per cent.

The following table shows results obtained.

Table No. 10.

HUMIDITY FACTOR

Flies sprayed with 0.5% Neotine nicotine sulphate solution.

Approx. Humidity	40%		75%		100%	
Repl.	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill	No. Flies Sprayed	% Kill
1	905	7.0	715	1.9	725	1.8
2	528	9.8	792	3.6	742	3.2
3	631	5.4	784	5.5	698	3.4
4	591	16.5	666	3.1	779	2.3
5	903	10.1	699	2.4	839	2.1
6	898	3.0	802	5.8	886	2.7
7	772	5.0	680	3.9	728	2.6
8	649	13.4	877	4.7	830	2.2
9	643	4.2	766	3.6	899	2.1
10	1094	5.2	1012	1.7	587	1.0
TOTAL	7614		7793		7713	
AVERAGE % KILL		7.38		3.76		2.53

GRAND TOTAL 23,120

Results indicate that the humidity at which flies were aged previous to spraying has very little if any effect upon toxicity. A slight difference is shown in mortality obtained with flies aged at 40 per cent humidity and flies aged at 100 per cent humidity, but this may be accounted for in part, if not entirely by the fact that the higher mortality obtained with flies aged at 40 per cent humidity was due to the poorer feeding conditions. Preliminary tests in this experiment resulted in a very high kill with flies aged at the forty per cent humidity which was discovered to be due to drying of the cone containing the banana. In obtaining the data shown for the lower humidities the feeding solutions were changed twice daily.

5. Culture Age Factor

As the emergence of flies from gum jar cultures may extend over a period of twenty days it was necessary to find out if aging cultures produce flies that vary in their susceptibility to nicotine. Accordingly, ten cultures were made up and the emergence from each culture for each day was sprayed separately with 0.5 per cent Neotine nicotine sulphate solution. Aging jars were prepared for each culture and corresponding numbers given to them. All flies were aged to three days before spraying. Results are shown in the following table.

Table No. 11

Culture Age Factor
Sprayed with 0.5% Neotine Solution

Jar. No.	1		2		3		4		5	
Age of Culture	No.Flies Sprayed	% Kill	No.Flies Sprayed	% Kill	No.Flies Sprayed	% Kill	No.Flies Sprayed	% Kill	No.Flies Sprayed	% Kill
13 days					205	7.3			232	14.2
14			735	7.2	370	6.2	297	5.7	192	8.9
15			609	6.2	426	6.1	154	7.1	426	8.0
16	379	13.9	756	5.9	268	5.2	256	3.5	282	7.1
17	273	6.6	364	2.4	357	4.5	310	18.4	435	6.2
18	315	8.9	337	3.8	333	4.5	108	23.1	521	2.3
19			185	6.5	445	6.3	197	4.5	423	5.2
20	236	8.5	264	3.8	337	5.9	75	4.0	445	5.6
21	209	14.3	194	7.2	172	8.1	56	10.7	306	7.5
22	84	7.1	126	3.9	261	4.9			309	3.2
23	122	13.9	174	5.7	179	7.2	215	4.2	234	2.1
24	36	16.6	179	15.6	259	5.8	145	3.4	102	4.8
25	55	9.1	85	3.5	121	4.9	100	4.0	61	11.5
26	134	8.9	196	3.6	284	8.5	82	1.2	79	3.8
27			114	9.6	187	14.4	70	5.7		
28	78	43.6	141	3.5	187	10.1	41	2.4		
29			141	4.1	194	2.6				
30					109	1.8	105	3.8		
31			482	4.2	120	5.0				
32										
33					125	8.8	110	4.5		
TOTAL	1921		4621		4939		2321		4047	
AVERAGE % KILL PER VIAL		11.9		6.7		6.3		7.3		6.0
AVERAGE NO. FLIES PER VIAL	76.8		100.4		109.7		92.8		112.4	

Jar. No.	6		7		8		9		10			
Age of Culture	No.Flies Sprayed	% Kill	No.Flies Sprayed	% Kill	No.Flies Sprayed	% Kill	No.Flies Sprayed	% Kill	No.Flies Sprayed	% Kill	TOTAL FLIES Sprayed	AVERAGE % Kill
13 days	210	11.4	221	17.6	333	10.7			258	6.6	1459	11.2
14	287	8.7	251	9.2	931	6.5	241	5.8	111	2.7	3414	6.9
15	39	7.7	329	2.7	433	5.1	186	2.1	189	6.3	2791	5.7
16	355	5.6	391	6.9	327	8.9	383	7.3	235	11.1	3632	7.4
17	386	6.2	683	3.2	570	4.4	584	3.2	548	8.2	4510	5.8
18	791	2.4	492	11.5	548	6.7	464	6.5			3909	6.0
19	752	4.4	356	3.1	753	3.1	541	4.8	429	2.8	4081	4.3
20	479	5.0	230	6.1	593	2.0	313	9.3			2972	5.3
21	459	4.8	264	1.5	134	6.8	617	2.8	466	5.8	2877	5.8
22	339	2.6	143	3.0	47	23.4	210	7.6	162	12.9	1681	6.2
23	410	2.7	24	4.2	164	12.3	498	7.0	425	3.3	2445	5.5
24	172	2.9	34	11.7	27	3.7	529	7.7	336	2.1	1819	6.1
25	122	6.5					222	7.7	268	4.5	1034	6.0
26	248	4.0	63	4.8			122	9.8	174	5.2	1382	5.8
27	135	5.9					65	20.0	90	7.8	661	10.6
28	124	14.5					124	18.6	44	4.5	739	13.8
29									54	25.9	389	6.4
30							82	7.3			296	4.1
31											602	4.3
32												
33											235	6.8
TOTAL	5308		3481		4860		5181		3789			
AVERAGE % KILL PER VIAL		4.9		6.5		5.8		6.3		6.0		
AVERAGE NO. FLIES PER VIAL	129.4		102.3		124.6		112.6		90.2			

Results contained in Table No. 11 show that there is little variation in per cent kill with flies sprayed at the beginning of emergence and flies sprayed at the end of emergence from cultures. The kill recorded for each day fluctuates only between 4.1 and 7.4 per cent with the exceptions of those for the first, fifteenth and sixteenth days of emergence.

The average per cent kill recorded by cultures for the whole emergence period shows little fluctuation, with the exception of the kill recorded for Culture No. 1. The emergence from this culture and Culture No. 4 was low and these cultures would have been discarded as a source of flies for general laboratory spraying.

SYMBOLS USED FOR NICOTINE-SOAP SOLUTIONS.

Concentration of Soaps Used.	Concentration of Nicotines Used. 0.5% Neotine	0.5% Britnico	0.5% Hyco	0.5% Nicofume
None	N0	B0	H0	Nf0
0.25% Sodium Fish Oil	N1	B1	H1	Nf1
0.5% Sodium Fish Oil	N2	B2	H2	Nf2
0.25% Potassium Fish Oil	N3	B3	H3	Nf3
0.5% Potassium Fish Oil	N4	B4	H4	Nf4
0.25% Sodium Oleate	N5	B5	H5	Nf5
0.5% Sodium Oleate	N6	B6	H6	Nf6
0.25% Potassium Oleate	N7	B7	H7	Nf7
0.5% Potassium Oleate	N8	B8	H8	Nf8
0.25% Triethanolamine Oleate	N9	B9	H9	Nf9
0.5% Triethanolamine Oleate	N10	B10	H10	Nf10

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VII COMPARATIVE TESTS EMPLOYING NICOTINE ADJUVANTS.

1. Experimental Outline. Four commercial brands of nicotines, consisting of the three nicotine sulphates "Neotine", "Britnico", "Hyco", and the alkaloid "Nicofume" were compared. Each brand was tested alone and in combination with 0.25 per cent and 0.5 per cent strengths of the following soaps: sodium and potassium fish oil soaps, sodium and potassium oleates and triethanolamine oleate. Check lots were included in each test but as the mortality was negligible, it was not considered necessary to correct the results obtained with the test solutions.

To eliminate error by possible early changes or interactions in the solutions, all replications were made up in sufficient amounts a month before being used. A slight amount of a brownish sediment appeared in the solutions containing the fish oil soaps which according to Mr. Hermon, did not alter their chemical or physical values. Atomization was not affected by the presence of the sediment in these solutions.

The forty-five different solutions were compared twenty-three times, the endeavor being made to use approximately one hundred flies for each solution per replication. It is believed the technique as previously described, has certain advantages over a technique previously investigated, in which each solution was compared by using about ten spraying tubes containing approximately fifteen flies each per replication. The advantages are suggested as follows: (1) There is little likelihood that the one hundred flies of one spraying tube vary to any appreciable extent in susceptibility to nicotine from the one hundred flies of another spraying tube, as both samples, obtained by the positive phototrophic responses of the flies, came from a population which was continually mixing of its own accord. (2) Flies were without a source of food for a short time only, as little time is required in admitting them to spraying tubes. (3) The transferring from spraying tubes to feeding tubes requires little individual handling. The flies do not seem to become stuck in the large spraying tubes as much as in the smaller spraying tubes and when they do, they can often be easily dislodged by blowing down through the spraying tube into the feeding tube.

2. Physical Properties of Solutions Tested. One month after the solutions to be tested were made up, the surface tension and pH value of each was determined by using a duNuoy tensiometer and potentiometer. In addition, the run-off point was determined by the following method. A type No2 Tattersfield atomizer was fixed in a horizontal position sixteen inches from an office wall file board, held in a vertical position and upon which was clamped a sheet of commercial waxed paper. The atomizer was connected with the air line of the spraying apparatus, previously described, and operated at a pressure equivalent to a height of fifteen inches of mercury, as

indicated by a manometer. The solution to be tested was contained in a graduate and held by hand to the suction pipe of the atomizer. The instant the spray film upon the waxed paper began to "run" the graduate was quickly lowered and the amount of solution used recorded. Three tests were made with each solution, the waxed paper being replaced each time.

Table No. 12 Number of flies sprayed using Neotine alone and in combination with soaps.

K = number of flies killed. T = number of flies sprayed.

	N0		N1		N2		N3		N4		N5		N6		N7		N8		N9		N10	
Rep.	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T
1	20	50	21	41	130	147	10	23	68	97	56	62	44	56	14	24	63	80	39	60	51	56
2	9	111	65	90	96	96	63	164	81	98	47	79	86	90	66	101	59	86	50	95	60	77
3	61	79	99	105	87	87	91	97	93	95	54	68	91	93	78	95	101	109	68	72	93	95
4	14	98	101	117	142	138	102	148	108	142	132	145	169	169	92	107	102	109	92	117	150	150
5	33	152	107	173	133	148	145	159	102	121	99	127	107	119	141	146	164	173	123	140	117	125
6	42	65	82	94	63	67	71	99	72	85	48	58	38	46	62	69	60	74	59	69	85	94
7	49	107	150	164	102	105	97	122	100	106	134	141	109	110	146	162	114	115	79	81	100	101
8	58	79	79	88	117	136	128	168	97	105	103	116	86	89	113	149	85	89	36	90	122	127
9	24	101	108	134	127	131	104	126	89	94	94	100	84	81	39	94	38	38	99	122	106	120
10	82	130	111	121	116	124	109	121	73	129	116	149	101	112	64	78	103	109	99	112	117	123
11	17	89	52	89	93	106	86	122	99	114	78	85	105	117	35	62	90	93	55	68	85	94
12	15	62	58	69	36	44	49	69	68	92	62	79	56	73	88	107	39	48	11	41	87	99
13	28	96	86	100	99	122	88	99	81	85	108	114	125	139	86	125	91	95	89	98	102	121
14	21	94	97	114	87	90	175	184	71	113	64	95	101	106	56	84	86	91	65	81	85	104
15	86	129	55	58	74	78	72	80	35	61	65	75	84	89	83	105	78	86	66	85	95	102
16	43	89	93	119	84	94	84	123	76	85	67	89	133	136	84	101	149	155	104	128	86	107
17	21	68	87	115	124	127	84	93	55	99	54	88	72	86	77	92	72	78	99	112	96	122
18	92	117	104	110	75	85	75	102	89	103	93	115	100	109	90	101	119	125	125	134	97	100
19	59	95	52	84	48	53	45	128	41	66	54	86	47	75	52	76	66	77	72	85	68	85
20	11	120	59	89	103	115	39	91	59	77	49	80	76	89	63	79	87	93	60	71	97	124
21	12	45	55	89	67	70	35	84	46	94	51	88	79	92	55	97	110	128	87	104	67	71
22	19	108	91	131	143	190	89	151	56	130	39	104	131	138	90	114	143	147	66	118	112	127
23	7	109	52	159	108	130	34	145	53	113	54	107	104	116	51	111	73	103	73	132	69	83

Table No. 13 Number of flies sprayed using Britnico alone and in combination with soaps.

K = number of flies killed. T = number of flies sprayed.

	B0		B1		B2		B3		B4		B5		B6		B7		B8		B9		B10	
Rep.	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T
1	4	87	50	78	84	95	49	78	74	87	47	74	71	75	52	62	98	105	51	62	63	92
2	2	75	35	57	87	98	27	52	42	70	30	48	47	51	94	110	73	83	95	109	83	92
3	15	91	84	104	102	108	115	127	72	83	77	88	115	116	95	108	91	101	115	118	98	113
4	24	179	91	100	128	132	126	156	153	164	122	136	138	138	70	111	171	174	115	131	137	137
5	18	100	90	95	153	158	55	69	112	114	92	103	133	133	138	148	75	85	74	76	89	95
6	5	56	39	50	75	83	52	75	54	114	63	74	65	77	64	72	71	79	75	82	62	71
7	4	101	101	132	119	121	65	82	108	111	107	108	113	118	110	116	127	132	93	108	139	151
8	10	113	131	140	115	117	78	97	109	123	60	67	77	81	55	86	88	91	102	110	94	120
9	33	103	170	189	117	119	117	140	97	105	123	138	128	131	156	169	142	147	68	77	25	36
10	19	108	62	92	116	121	84	109	75	101	135	142	83	102	20	36	117	123	106	112	91	116
11	1	90	61	71	74	75	52	75	62	67	78	88	81	85	46	61	76	82	63	79	62	83
12	9	79	55	78	39	55	29	47	70	82	57	75	50	65	46	81	50	70	58	67	48	72
13	25	127	82	110	127	132	87	125	132	150	50	62	74	93	126	133	83	87	98	110	66	71
14	9	84	94	130	96	100	49	57	62	77	103	115	68	77	63	87	98	109	124	128	89	125
15	15	91	125	147	97	97	64	75	63	86	93	101	100	104	51	71	86	88	92	94	95	112
16	16	119	72	81	76	84	70	103	102	115	77	91	119	121	68	86	97	107	77	84	82	90
17	17	73	101	119	115	122	89	105	70	92	80	86	79	91	86	95	77	79	77	90	51	60
18	21	66	101	104	55	60	56	66	114	147	73	83	172	174	90	107	77	83	105	114	101	113
19	40	88	41	64	120	126	34	74	60	82	52	71	45	49	65	103	76	104	86	105	73	104
20	2	78	89	140	74	93	75	117	62	132	115	124	123	146	92	116	77	96	113	128	24	78
21	4	116	101	101	127	162	42	90	78	123	77	109	77	113	57	115	83	115	82	97	78	128
22	10	101	40	102	132	157	47	144	62	123	70	108	78	84	87	141	96	112	98	149	44	115
23	3	111	51	117	123	149	65	114	37	85	63	135	84	114	57	132	107	124	107	138	65	111

Table No. 14 Number of flies sprayed using Hyco alone and in combination with soaps.

K = number of flies killed. T = number of flies sprayed.

	H0		H1		H2		H3		H4		H5		H6		H7		H8		H9		H10	
Rep.	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T
1	6	116	7	123	18	124	36	133	48	113	60	101	83	116	18	82	87	137	10	108	118	131
2	3	72	6	81	76	82	9	79	16	81	16	64	53	114	14	122	25	78	9	82	39	81
3	38	77	76	129	106	109	45	79	38	89	81	87	60	115	38	90	79	90	24	58	90	102
4	16	146	30	87	143	154	60	143	94	148	86	112	78	91	22	95	89	98	44	144	97	139
5	15	70	60	100	76	81	86	174	89	122	93	113	90	135	33	96	99	131	49	107	115	128
6	6	79	21	57	47	75	25	70	37	73	31	83	49	71	7	65	47	87	5	42	44	73
7	29	114	40	114	111	114	58	102	68	129	62	102	123	144	87	137	74	95	53	112	98	125
8	17	85	40	89	96	104	44	56	61	79	80	106	112	151	11	84	74	134	27	111	71	127
9	8	72	26	51	60	67	29	48	95	131	59	97	55	68	19	82	111	136	26	64	59	72
10	14	100	18	114	107	143	86	137	70	122	68	132	79	145	31	118	101	157	31	130	119	137
11	13	106	23	83	74	87	24	73	18	48	20	60	48	85	15	91	45	76	19	108	25	53
12	9	95	23	73	30	52	16	61	25	77	38	73	34	56	14	75	23	53	13	45	19	64
13	38	106	40	109	123	128	41	105	69	110	73	97	102	136	62	112	89	115	51	126	83	119
14	20	55	33	84	59	77	20	53	42	73	58	75	46	61	17	83	35	68	43	58	82	89
15	13	91	45	96	41	82	53	119	63	131	53	72	53	68	37	88	55	82	18	72	51	86
16	11	117	39	88	122	138	28	96	68	102	68	99	42	59	18	91	56	102	10	99	70	101
17	15	124	16	82	95	118	26	82	93	128	73	125	51	103	30	115	55	99	16	83	80	90
18	99	128	71	96	121	125	68	91	82	89	82	119	88	100	107	116	102	111	60	82	94	101
19	14	93	18	89	89	98	31	113	79	126	48	117	65	117	21	118	73	23	46	122	88	120
20	7	16	14	88	75	106	15	89	46	113	25	67	53	103	0	85	46	90	13	83	73	103
21	13	94	28	95	32	72	8	64	45	75	17	90	67	108	24	133	43	87	21	112	37	101
22	2	156	119	118	49	83	29	120	28	88	39	112	52	83	13	73	44	94	19	97	64	109
23	13	106	10	129	108	163	10	113	25	98	23	131	20	137	7	127	34	108	14	145	56	121

Table No. 15 Number of flies sprayed using Nicofume alone and in combination with soaps.

K = number of flies killed. T = number of flies sprayed.

	Nf0		Nf1		Nf2		Nf3		Nf4		Nf5		Nf6		Nf7		Nf8		Nf9		Nf10	
Rep.	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T	K	T
1	68	132	196	202	135	140	125	129	136	145	142	171	131	153	47	50	46	46	74	76	40	45
2	86	102	154	186	119	147	71	86	136	149	92	114	89	95	91	101	140	143	75	97	82	101
3	92	110	89	93	89	97	105	109	85	90	77	77	77	78	70	70	84	86	86	88	95	99
4	144	151	122	123	133	136	99	110	127	153	112	127	124	127	131	143	77	77	120	126	112	114
5	130	134	134	140	92	98	82	86	102	110	101	143	105	113	88	98	87	92	92	100	113	115
6	75	89	66	71	56	57	76	84	57	83	70	90	73	77	72	80	54	57	66	70	87	92
7	78	101	110	110	92	94	93	97	91	94	122	126	86	86	86	102	86	91	95	96	87	91
8	42	57	63	95	104	105	78	82	75	77	81	98	89	97	75	76	92	102	70	90	109	113
9	75	87	88	92	92	92	68	91	45	57	83	87	79	79	82	85	78	86	68	77	85	92
10	47	89	110	119	118	125	19	25	79	98	24	29	92	109	118	122	130	134	77	106	73	87
11	101	110	98	102	132	137	65	92	71	100	121	131	110	111	87	116	91	96	89	106	96	101
12	60	72	66	77	44	55	54	64	44	54	50	63	58	65	57	59	55	61	79	90	49	58
13	101	110	134	146	120	120	81	93	95	110	109	112	96	108	114	120	124	125	95	104	99	102
14	104	121	108	116	69	75	82	92	83	86	56	66	65	73	91	108	81	88	73	76	73	80
15	59	83	90	106	90	98	113	121	101	122	106	113	73	75	61	81	94	96	113	115	104	105
16	73	77	105	121	134	137	124	130	112	123	93	100	88	113	100	123	81	96	100	103	102	105
17	73	114	106	120	92	98	84	99	53	87	102	111	80	89	85	96	103	111	61	81	103	109
18	87	104	126	133	115	118	72	74	104	113	101	106	93	97	119	126	117	118	98	102	80	85
19	75	101	101	139	71	87	108	135	85	127	69	76	81	113	73	104	94	110	24	25	88	105
20	20	80	57	79	46	81	90	106	31	98	95	113	99	105	81	97	126	137	109	115	87	104
21	66	95	111	119	96	97	75	92	78	94	71	76	65	71	80	110	82	89	95	105	69	94
22	32	75	65	83	133	152	88	125	13	60	86	116	137	182	116	138	58	76	103	115	92	104
23	109	142	101	128	101	141	74	176	69	112	151	168	121	147	117	130	117	141	59	119	80	121

Table No. 16 Replicate 1.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	40.0	51.2	88.5	36.3	70.2	90.3	78.6	58.4	78.8	65.0	91.1
Britnico	4.6	61.1	88.4	62.9	85.0	63.5	94.6	83.9	93.4	82.3	68.4
Hyco	5.2	5.7	8.0	27.1	42.5	59.3	71.5	21.9	63.5	9.3	90.2
Nicofume	51.5	97.2	96.5	97.0	93.8	83.2	85.7	94.0	100.0	97.4	89.0

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Per cent kill expressed in Angles of Equal Information (θ)...(Bliss, Ref. No. 2)

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	39.23	45.69	70.18	37.05	56.91	71.85	62.44	49.84	62.58	53.73	72.64
Britnico	12.39	43.19	70.09	52.48	67.21	52.83	76.56	66.34	75.11	65.12	55.80
Hyco	13.18	13.81	16.43	31.37	40.69	50.36	57.73	27.90	52.83	17.76	71.76
Nicofume	45.86	80.37	79.22	80.02	75.58	65.80	67.78	75.82	90.00	80.72	70.63

Table No. 17 Replicate 2.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	8.1	72.2	100.0	38.4	82.7	59.5	95.6	65.3	68.7	52.7	78.0
Britnico	26.7	61.4	88.9	52.0	60.0	62.5	92.2	85.5	88.0	87.2	90.3
Hyco	4.2	7.5	92.6	11.4	19.6	25.0	46.5	11.5	32.1	10.9	48.2
Nicofume	84.3	82.8	81.0	82.5	91.4	80.6	93.7	90.1	97.8	77.4	81.2

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Per Cent Kill expressed in Angles of Equal Information (θ) .

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	16.54	58.18	90.00	38.29	65.42	50.48	77.89	53.91	55.98	46.55	62.08
Britnico	31.11	51.59	70.54	46.15	50.77	52.24	73.78	67.62	69.73	69.04	71.85
Hyco	11.83	15.89	74.21	19.73	26.28	30.00	42.99	19.82	34.51	19.28	43.97
Nicofume	66.66	65.50	64.16	65.27	72.95	63.87	75.46	71.66	81.47	61.62	64.30

Table No. 18 Replicate, 3.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	77.2	94.3	100.0	93.8	97.9	79.4	97.8	82.2	92.7	94.5	97.9
Britnico	16.5	80.5	94.5	90.5	86.8	87.6	99.2	88.0	90.0	97.5	86.7
Hyco	49.4	58.9	97.4	57.0	38.8	93.2	52.2	42.3	82.7	41.4	88.2
Nicofume	83.6	95.8	91.7	96.4	94.5	100.0	98.8	100.0	97.7	97.8	96.0

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Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	61.48	76.19	90.00	75.58	81.67	63.01	81.47	65.05	74.32	76.44	81.67
Britnico	23.97	63.79	76.44	72.05	68.70	69.38	84.87	69.73	71.56	80.90	68.61
Hyco	44.66	50.13	80.72	49.02	38.53	74.88	46.26	40.57	65.42	40.05	69.91
Nicofume	66.11	78.17	73.26	79.06	76.44	90.00	83.71	90.00	81.28	81.47	78.46

Table No. 19 Replicate 4.

Kill expressed in per centage.

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	14.3	86.3	95.9	68.9	76.1	91.0	100.0	86.0	93.6	78.7	100.0
Britnico	13.4	91.0	97.0	81.0	93.4	89.9	100.0	63.1	98.3	87.9	100.0
Hyco	10.9	34.5	93.0	42.0	63.5	76.7	85.7	23.2	90.7	30.5	69.7
Nicofume	95.4	91.1	97.8	90.0	83.1	83.3	97.7	91.8	100.0	95.3	98.3

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Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	22.22	68.28	78.32	56.11	60.73	72.54	90.00	68.03	75.35	62.51	90.00
Britnico	21.47	72.54	80.02	64.16	75.11	71.47	90.00	52.59	82.51	69.64	90.00
Hyco	19.28	35.97	74.66	40.40	52.83	61.14	67.78	28.79	72.24	33.52	56.60
Nicofume	77.61	72.64	81.47	71.56	65.73	65.88	81.28	73.46	90.00	77.48	82.51

Table No. 20 Replicate 5.

Kill expressed in per centage.

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	21.7	61.8	96.4	91.2	84.3	78.0	89.9	96.6	94.8	88.0	93.7
Britnico	18.0	94.7	97.0	79.8	98.4	89.5	100.0	93.3	88.2	97.4	93.7
Hyco	21.2	60.0	93.9	49.5	73.0	82.3	67.7	34.4	75.6	45.8	90.0
Nicofume	97.1	95.8	93.9	95.4	92.7	70.7	93.0	89.7	94.6	92.0	98.3

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Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	27.76	51.83	79.06	72.74	66.66	62.03	71.47	79.37	76.82	69.73	75.46
Britnico	25.10	76.69	80.02	63.29	82.73	71.09	90.00	75.00	69.91	80.72	75.46
Hyco	27.42	50.77	75.70	44.71	58.69	65.12	55.37	35.91	60.40	42.59	71.56
Nicofume	80.19	78.17	75.70	77.61	74.32	57.23	74.66	71.28	76.56	73.57	82.51

Table No. 21 Replicate 6.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	64.6	87.2	94.0	71.7	84.8	82.8	82.7	89.9	81.1	85.6	90.3
Britnico	8.9	78.0	90.3	69.4	47.4	85.3	84.5	88.9	89.7	91.5	87.4
Hyco	76.0	37.0	62.7	35.7	50.6	37.4	69.0	10.7	54.1	11.9	60.3
Nicofume	84.3	92.9	98.3	90.5	63.7	77.7	94.7	90.0	94.7	94.3	94.6

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Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	53.49	69.04	75.82	57.86	67.05	65.50	65.42	71.47	64.23	67.70	71.85
Britnico	17.36	62.03	71.85	56.42	43.51	67.45	66.81	70.54	71.47	73.05	69.21
Hyco	60.67	37.47	52.36	36.69	45.34	7.70	56.17	19.09	47.35	20.18	50.94
Nicofume	66.66	74.55	82.51	72.05	52.95	61.82	76.69	76.19	76.69	71.56	76.56

Table No. 22 Replicate 7.

Kill expressed in per centage.

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	45.8	91.5	97.1	79.5	94.4	95.1	99.1	90.1	99.2	97.6	99.1
Britnico	3.9	76.5	98.4	79.3	97.5	99.0	95.7	94.9	96.3	86.2	92.1
Hyco	25.2	35.1	96.5	56.7	52.8	60.7	85.5	64.3	77.9	47.4	78.4
Nicofume	77.2	100.0	97.8	95.8	96.8	96.9	100.0	84.3	94.6	99.0	95.6

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Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	42.59	73.05	80.19	63.08	76.31	77.21	84.56	71.66	84.87	81.09	84.56
Britnico	11.39	61.00	82.73	62.94	80.90	84.26	78.03	76.95	78.91	68.19	73.68
Hyco	30.13	36.33	79.22	48.85	46.61	51.18	67.62	53.31	61.96	43.51	62.31
Nicofume	61.48	90.00	81.47	78.17	79.69	79.86	90.00	66.66	76.56	84.26	77.89

Table No. 23 Replicate 8.

Kill expressed in per centage.

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	73.4	89.8	86.0	76.2	92.4	88.8	96.7	75.9	95.6	40.0	96.2
Britnico	8.8	93.5	98.4	80.5	88.7	89.5	95.0	63.8	96.7	90.1	77.3
Hyco	20.0	45.0	92.3	78.6	77.3	75.5	80.8	13.1	55.2	24.3	55.9
Nicofume	73.7	67.3	99.2	95.2	97.4	82.7	91.7	98.7	90.2	77.9	96.5

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Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	58.95	71.37	68.03	60.80	74.00	70.45	79.53	60.60	77.89	39.23	78.76
Britnico	17.26	75.23	82.73	63.79	70.36	71.09	77.08	53.01	79.53	72.54	61.55
Hyco	26.56	43.13	73.89	25.55	61.55	60.33	64.01	21.22	47.98	29.53	48.39
Nicofume	59.15	55.12	84.87	77.34	80.72	65.42	73.26	83.45	71.76	61.96	79.22

Table No. 24 Replicate 9.

Kill expressed in per centage.

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	23.1	80.6	96.9	82.6	94.7	94.0	92.3	41.5	100.0	81.2	88.4
Britnico	32.0	90.0	98.4	83.6	92.4	89.3	97.7	92.3	96.7	88.3	69.5
Hyco	11.1	51.0	91.0	60.5	72.5	60.8	80.9	23.2	81.8	40.7	82.0
Nicofume	86.3	95.6	100.0	74.7	79.0	95.4	100.0	96.5	90.7	88.4	92.4

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Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	29.13	63.87	79.86	65.35	76.69	75.82	73.89	40.11	90.00	64.30	70.09
Britnico	34.45	71.56	82.73	66.11	74.00	70.91	81.28	73.89	79.53	70.00	56.48
Hyco	19.46	45.57	72.54	51.06	58.37	51.24	64.08	28.79	64.75	39.64	64.90
Nicofume	68.28	77.89	90.00	59.80	62.72	77.61	90.00	79.22	72.24	70.09	74.00

Table No. 25 Replicate 10.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	63.1	91.7	93.5	90.1	56.6	77.9	90.2	82.1	94.5	81.2	95.2
Britnico	17.6	67.5	96.0	77.0	74.3	95.2	86.5	55.6	95.3	94.7	77.5
Hyco	14.0	15.8	75.0	62.6	57.4	51.5	54.5	26.2	64.4	23.8	87.0
Nicofume	52.8	92.5	94.5	71.1	80.6	96.3	84.3	96.7	92.2	72.6	84.0

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	52.59	73.26	75.23	71.66	48.79	61.96	71.76	65.05	76.44	64.30	77.34
Britnico	24.80	55.24	78.46	61.34	59.54	77.34	64.52	48.22	77.48	76.69	61.68
Hyco	21.97	23.42	60.00	52.30	49.26	45.86	47.58	30.79	53.37	29.20	68.87
Nicofume	46.61	74.11	76.44	57.48	63.87	78.91	66.66	79.53	73.78	58.44	66.42

Table No. 26 Replicate 11.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	19.1	58.4	87.7	70.5	86.8	91.8	89.8	56.5	96.8	80.9	90.3
Britnico	1.1	85.9	98.7	69.4	92.5	88.6	95.3	75.5	92.7	79.8	74.7
Hyco	12.3	27.7	85.1	32.9	37.5	33.3	56.5	16.5	59.4	17.6	47.2
Nicofume	91.9	96.1	96.4	70.7	71.0	92.4	99.2	75.0	94.7	84.0	95.1

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	25.92	49.84	69.47	57.10	68.70	73.36	71.37	48.73	76.69	64.08	71.85
Britnico	6.02	67.94	83.45	56.42	74.11	70.27	77.48	60.33	74.32	63.29	59.80
Hyco	20.53	31.76	67.29	35.00	37.76	35.24	48.73	23.79	50.42	24.80	43.39
Nicofume	73.46	78.61	79.06	57.23	57.42	74.00	84.87	60.00	76.69	66.42	77.21

Table No. 27 Replicate 12.

Kill expressed in per centage.

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	24.2	84.0	81.9	71.0	73.9	78.5	76.8	82.2	81.3	26.8	88.0
Britnico	11.4	70.5	71.0	61.7	85.4	76.0	77.0	56.8	71.5	86.5	66.7
Hyco	9.4	31.6	57.7	26.2	32.4	52.8	60.7	18.7	43.4	28.9	29.7
Nicofume	83.4	85.7	80.0	84.4	81.5	79.4	89.3	96.6	90.3	87.8	84.5

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	29.47	66.42	64.82	57.42	59.28	62.37	61.21	65.05	64.38	31.18	69.73
Britnico	19.73	57.10	57.42	51.77	67.54	60.67	61.34	48.91	57.73	68.44	74.76
Hyco	17.85	34.20	49.43	30.79	34.70	46.61	51.18	25.62	41.21	32.52	33.02
Nicofume	65.96	67.78	63.44	66.74	64.52	63.01	70.91	79.37	71.85	69.56	66.81

Table No. 28 Replicate 13.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	29.2	86.0	81.1	88.9	95.3	94.8	89.9	68.8	95.8	90.8	84.3
Britnico	19.7	74.5	96.2	69.6	88.0	80.6	79.7	94.7	90.4	89.0	93.0
Hyco	35.9	37.0	96.1	39.0	62.7	75.4	70.2	55.4	77.4	40.5	69.7
Nicofume	91.0	91.2	100.0	87.2	86.3	97.4	88.7	95.3	99.3	91.4	97.1

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	32.71	68.03	64.23	70.54	77.48	76.82	71.47	56.04	78.17	72.34	66.66
Britnico	26.35	59.67	78.76	56.54	69.73	63.87	63.22	76.69	71.95	70.63	74.66
Hyco	36.81	37.47	78.61	38.65	52.36	60.27	56.91	48.10	61.62	39.52	56.60
Nicofume	72.54	72.74	90.00	69.04	68.28	80.72	70.36	77.48	85.20	72.95	80.19

Table No. 29. Replicate 14.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	22.3	85.1	96.6	95.1	62.8	67.4	95.3	66.6	94.5	80.3	81.6
Britnico	10.7	72.3	96.0	86.0	80.5	89.5	88.4	72.5	90.0	97.0	71.2
Hyco	36.4	39.3	76.6	37.8	57.5	77.4	75.5	20.4	51.4	74.2	92.2
Nicofume	86.0	93.3	92.0	89.2	96.5	84.7	89.1	84.3	92.1	96.0	91.3

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	28.18	67.29	79.37	77.21	52.42	55.18	77.48	54.70	76.44	63.65	64.60
Britnico	19.09	58.24	78.46	68.03	63.79	71.09	70.09	58.37	71.56	80.02	57.48
Hyco	37.11	38.82	61.07	37.94	49.31	61.62	60.33	26.85	45.80	59.47	73.78
Nicofume	68.03	75.00	73.57	70.81	79.22	66.97	70.72	66.66	73.68	78.46	72.84

Table No. 30 Replicate 15.

Kill expressed in per centage

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	66.6	94.8	94.9	90.0	57.4	86.7	94.4	78.1	90.7	77.7	93.2
Britnico	16.5	85.0	100.0	85.4	73.4	92.0	96.1	71.9	97.8	97.9	84.8
Hyco	14.3	46.9	50.0	44.5	48.0	73.6	78.0	42.1	67.1	14.5	59.4
Nicofume	71.1	84.8	91.8	93.4	82.9	93.8	97.4	75.4	97.9	92.0	99.1

....

Per Cent Kill expressed in Angles of Euqal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	54.70	76.82	76.95	71.56	49.26	68.61	76.31	62.10	72.24	61.82	74.88
Britnico	23.56	67.21	90.00	67.54	58.95	73.57	78.61	57.99	81.47	81.67	67.05
Hyco	22.22	43.22	45.00	41.84	43.85	59.08	62.03	40.46	55.00	22.38	50.42
Nicofume	57.48	67.05	73.36	75.11	65.57	75.58	80.72	60.27	81.67	73.57	84.56

Table No. 31 Replicate 16.

Kill expressed in per centage.

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	48.3	78.2	89.4	68.3	89.4	75.3	97.8	83.2	96.1	81.3	80.4
Britnico	13.5	88.9	90.5	68.0	88.8	84.6	98.5	79.1	90.6	91.7	91.2
Hyco	9.4	44.4	88.4	29.2	66.6	68.7	71.3	19.8	54.9	10.1	69.3
Nicofume	94.7	86.8	97.9	95.4	91.2	93.0	77.4	81.4	84.3	97.1	97.2

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	44.03	62.17	71.00	55.73	71.00	60.20	81.47	65.80	78.61	64.38	63.72
Britnico	21.56	70.54	72.05	55.55	70.45	66.89	82.96	62.80	72.15	73.26	72.74
Hyco	17.85	41.78	70.09	32.71	54.70	55.98	57.61	26.42	36.21	18.53	56.35
Nicofume	76.69	68.70	81.67	77.61	72.74	74.66	61.62	64.45	66.66	80.19	80.37

Table No. 32 Replicate 17.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	30.9	75.6	97.6	90.3	55.6	61.4	83.8	83.7	92.4	88.4	78.7
Britnico	23.3	84.1	94.4	84.7	76.0	93.0	86.8	90.6	97.5	85.6	85.0
Hyco	12.1	19.6	80.5	31.7	72.6	58.9	49.5	26.1	55.7	14.0	88.9
Nicofume	64.0	83.4	93.7	84.4	60.9	92.7	89.9	88.4	93.7	75.3	94.5

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	33.77	60.40	81.09	71.85	48.22	51.59	66.27	66.19	74.00	70.09	62.51
Britnico	28.86	66.50	76.31	66.97	60.67	74.66	68.70	72.15	80.90	67.70	67.21
Hyco	20.36	26.28	63.79	34.27	58.44	50.13	44.71	30.72	48.27	21.97	70.54
Nicofume	53.13	65.96	75.46	66.74	51.30	74.32	71.47	70.09	75.46	60.20	76.44

Table No. 33 Replicate 18.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	78.6	94.5	88.2	73.5	86.4	80.9	91.8	89.2	95.2	93.4	97.0
Britnico	31.9	97.2	91.7	84.9	77.5	88.0	99.0	84.2	92.8	91.9	89.4
Hyco	77.3	74.0	96.8	74.7	92.2	68.9	88.0	92.4	92.0	73.2	93.1
Nicofume	83.7	94.8	97.6	92.3	92.2	95.4	95.7	94.4	99.3	96.1	94.2

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	62.44	76.44	69.91	59.02	68.36	64.08	73.36	70.81	77.34	75.11	80.02
Britnico	34.39	80.37	73.26	67.13	61.68	69.73	84.26	66.58	77.44	73.46	71.00
Hyco	61.55	59.34	79.69	59.80	73.78	56.11	69.73	74.00	73.57	58.82	74.77
Nicofume	66.19	76.82	81.09	73.89	73.78	77.61	78.03	76.31	85.20	78.61	76.06

Table No. 34 Replicate 19.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	62.1	61.9	90.5	35.2	62.1	62.8	62.7	68.5	85.7	84.7	80.0
Britnico	45.5	64.1	95.3	37.5	73.3	73.4	92.0	63.2	73.1	81.9	70.2
Hyco	15.0	20.3	90.7	27.4	62.7	41.0	55.6	17.8	59.4	37.7	73.2
Nicofume	74.2	72.7	81.6	80.0	66.8	90.7	71.7	70.2	80.8	96.0	83.8

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Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	52.00	51.88	72.05	36.39	52.00	52.42	52.36	55.86	67.78	66.97	63.44
Britnico	42.42	43.19	77.48	37.76	58.89	58.95	73.57	52.65	58.76	64.82	56.91
Hyco	22.79	26.78	72.24	31.56	52.36	39.82	48.22	24.95	50.42	37.88	58.82
Nicofume	59.47	58.50	64.60	63.44	54.82	72.24	57.86	56.91	64.01	78.46	66.27

Table No. 35 Replicate 20.

Kill expressed in per centage.

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	9.2	66.3	89.6	42.9	76.7	61.3	85.4	78.5	93.5	84.5	78.3
Britnico	2.5	63.6	79.5	64.1	47.0	92.9	84.2	79.3	80.3	88.4	30.8
Hyco	6.0	16.0	70.7	16.8	40.7	37.4	51.4	0	51.1	15.7	70.8
Nicofume	25.0	72.2	56.8	84.9	31.6	84.1	94.3	83.5	92.0	94.8	83.7

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	17.66	54.51	71.19	40.92	61.14	51.53	67.54	62.37	75.23	66.81	62.24
Britnico	9.10	52.89	63.08	43.19	43.28	74.55	66.58	62.94	63.65	70.09	33.71
Hyco	14.18	23.58	57.23	24.20	39.64	37.70	45.80	0	45.63	23.34	57.29
Nicofume	30.00	58.18	48.91	67.13	34.20	66.50	76.19	66.03	73.57	76.82	66.19

Table No. 36 Replicate 21.

Kill expressed in per centage.

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	26.6	61.8	92.8	41.7	48.9	58.0	85.9	56.8	85.9	83.6	94.4
Britnico	3.4	100.0	78.5	46.7	63.4	70.7	68.2	49.6	72.2	84.5	60.8
Hy co	13.8	29.5	44.5	12.5	60.0	18.9	62.1	18.1	49.4	18.7	36.7
Nicofume	69.5	93.4	99.0	81.5	83.0	93.4	91.5	72.7	92.3	90.5	73.5

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps										
	0	1	2	3	4	5	6	7	8	9	10
Neotine	31.05	51.83	74.44	40.22	44.37	49.60	67.94	48.91	67.94	66.11	76.31
Britnico	10.31	90.00	62.37	43.11	52.77	57.23	55.67	44.77	58.18	66.81	51.24
Hy co	21.81	32.90	41.84	20.70	50.77	25.77	52.00	25.18	44.66	25.62	37.29
Nicofume	56.48	75.11	84.26	64.52	65.65	75.11	73.05	58.50	73.89	72.05	59.02

Table No. 37 Replicate 22.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	17.6	69.5	75.3	58.9	43.1	29.1	94.9	78.9	97.3	55.9	88.4
Britnico	9.9	39.2	84.2	32.6	50.4	64.7	92.9	61.7	85.6	65.7	38.2
Hy co	1.3	16.1	59.0	24.2	32.6	34.8	62.7	17.8	46.8	19.6	58.7
Nicofume	42.6	78.4	87.6	70.4	21.7	74.1	75.3	84.2	76.4	89.6	88.5

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	24.80	56.48	60.20	50.13	41.3	32.65	76.95	62.65	80.54	48.39	70.9
Britnico	18.34	38.76	66.58	34.82	45.23	53.55	74.55	51.71	67.70	54.15	38.17
Hy co	6.55	23.66	50.18	29.47	34.82	36.15	52.36	24.95	43.17	26.28	50.01
Nicofume	40.74	62.31	69.38	57.04	27.76	59.41	60.20	66.58	60.94	71.19	70.18

Table No. 38 Replicate 23.

Kill expressed in per centage.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	6.4	32.7	83.1	23.4	46.9	50.5	89.7	45.9	70.9	55.3	83.2
Britnico	2.7	43.5	82.5	56.9	43.5	46.6	73.6	43.2	86.4	77.6	58.5
Hyco	12.3	7.8	66.4	8.8	25.6	17.5	14.6	5.5	31.4	9.7	46.3
Nicofume	76.7	71.7	71.7	42.0	61.6	89.9	82.4	90.1	83.0	49.6	66.1

....

Per Cent Kill expressed in Angles of Equal Information (θ).

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10
Neotine	14.65	34.88	65.73	28.93	43.22	45.29	71.28	42.65	57.35	48.04	65.80
Britnico	9.46	41.27	65.27	48.97	41.27	43.05	59.08	41.09	68.36	61.75	49.89
Hyco	20.35	16.22	54.57	17.26	30.40	24.73	22.46	13.56	34.08	18.15	42.88
Nicofume	61.14	57.86	57.86	40.40	51.71	71.47	65.20	71.66	65.65	44.77	54.39

Table No. 39. Variation Due to Nicotines.

Sum of the Angles of Equal Information for each nicotine for the entire series.

Neotine	15957.48
Britnico	15757.14
Hyco	11008.90
Nicofume	17875.36
TOTAL	60598.88
Total sum of squares	943,653,003.27

Table No. 40 Variation Due to Soaps.

Sum of the Angles of Equal Information for each soap for twenty-three replications.

Soap	Neotine	Britnico	Hyco	Nicofume	TOTAL
0	855.36	488.90	595.30	1419.92	3359.48
1	1427.75	1426.54	788.50	1621.14	5263.93
2	1707.14	1720.10	1450.76	1731.76	6609.76
3	1315.54	1306.53	833.87	1568.06	5024.00
4	1410.71	1441.19	1091.04	1471.94	5414.88
5	1414.55	1526.14	1087.02	1638.00	5665.71
6	1673.44	1699.04	1241.66	1700.70	6314.84
7	1386.95	1410.87	690.97	1636.95	5125.74
8	1685.19	1656.91	1190.87	1744.81	6277.78
9	1424.55	1521.98	724.54	1649.05	5420.12
10	1656.30	1458.94	1314.37	1683.03	6112.64

TOTAL 60,598.88

SUM OF SQUARES OF TOTALS 341,648,768.47

Table No. 41 Variation Due to Interaction of Soap and Nicotine.

Sum of the Angles of Equal Information for each corresponding nicotine and soap for twenty-three replications.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10	Total
Neotine	855.36	1427.75	1707.14	1315.54	1410.71	1414.55	1673.44	1386.95	1685.19	1424.55	1656.30	15,957.48
Britnico	488.90	1426.54	1720.10	1306.53	1441.19	1526.14	1699.04	1410.87	1656.91	1621.98	1458.94	15,757.14
Hyco	595.30	788.50	1450.76	833.87	1091.04	1087.02	1241.67	690.97	1190.87	724.54	1314.37	11,008.90
Nicofume	1419.92	1621.14	1731.76	1568.06	1471.94	1638.00	1700.70	1636.95	1744.81	1649.05	1683.03	17,875.36

TOTAL 60,598.88

TOTAL SUM OF SQUARES 88,428,658.55

Table No. 42 Sum of Squares for each Variant.

$$S(x)^2 - \frac{(S(x))^2}{n}$$

Total Sum of Squares $= 3,963,314.03 - \frac{(60,598.88)^2}{1012}$

$= 334,831.57$

....

$$\frac{S(\text{items})^2}{(\text{no. of values composing each item})} - \frac{(S(x))^2}{n}$$

Nicotines $= \frac{943,653,003.27}{253} - \frac{3,672,024,257.25}{1012}$

$= 101,371.24$

Soaps $= \frac{341,648,768.47}{92} - \frac{3,672,024,257.25}{1012}$

$= 85,145.04$

Interaction of Soaps and Nicotines $= \frac{88,428,658.55}{23} - \frac{3,672,024,257.25}{1012}$

$= 29,725.54$

....

Table No. 43 Analysis for Variance.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance or Mean Square	Standard Deviation	$\frac{1}{2}$Log Mean Square	z Value	z Value for 5% pt.	1% pt.
Nicotines	3	101,371.24	33,790.41		5.2140	2.8099	.4787	.6651
Soaps	10	85,145.04	8,514.50		4.5248	2.1207	.305	.4256
Interaction of Soap and Nicotine	30	29,725.54	990.85		3.4493	1.0452	0	0
Remainder	968	118,589.75	122.51	11.07	2.4041			
Total	1011	334,831.57	331.19		2.9014			

Table No. 44 Average Kill for each Soap, Expressed in Angles of Equal Information (θ).

Soap No.	Soap	Average Kill
2	0.5% Sodium Fish Oil	70.02
8	0.5% Potassium Oleate	67.78
6	0.5% Sodium Oleate	67.28
10	0.5% Triethanolamine Oleate	65.25
5	0.25% Sodium Oleate	61.21
9	0.25% Triethanolamine Oleate	58.15
4	0.5% Potassium Fish Oil	58.04
1	0.25% Sodium Fish Oil	56.73
7	0.25% Potassium Oleate	55.55
3	0.25% Potassium Fish Oil	54.47
0	None	36.51

Standard Deviation of one observation = 11.07

Standard Deviation of a mean of 92 observations = 1.154

Standard Deviation of a difference = 1.63

Difference required for significance ($P = .05$) = 3.19

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There is no significant difference among the first four soaps, nor is there a significant difference among the last six. The first four soaps, however, Nos. 2, 8, 6 and 10 are all significantly better than the remainder.

Table No. 45. Average Kill for each Nicotine, Expressed in Angles of Equal Information (θ).

Nicotine	Average Kill
Nicofume	69.12
Neotine	62.28
Britnico	61.39
Hyco	43.98

Standard Deviation of one observation = 11.07

Standard Deviation of a mean of 253 observations = 0.7

Standard Deviation of a difference = 0.99

Difference required for significance (P = .05) = 1.94

....

There is a significant difference between Nicofume and Neotine, and between Britnico and Hyco, but none between Neotine and Britnico.

....

Taking the experiment as a whole, the analysis of variance shows that the difference in nicotines is significant and the difference in soaps is significant.

Table No. 46 Physical Properties of Solutions Tested.

pH Value

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10	Mean.
Neotine	5.84	6.91	7.06	6.91	7.56	6.69	7.28	7.11	7.20	6.60	6.89	6.91
Britnico	6.18	6.94	7.37	7.03	7.70	6.91	7.37	7.28	7.49	7.03	7.28	7.14
Hyco	4.66	5.96	6.77	6.72	7.03	6.55	6.72	6.52	7.03	6.35	6.52	6.44
Nicofume	9 -	9 -	9 -	9 -	9 -	8.97	8.80	9 -	9 -	8.78	8.55	8.92

....

Surface Tension (dynes/cc.)

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10	Mean
Neotine	60.16	35.26	33.84	38.54	37.13	33.84	32.43	31.96	31.49	32.90	31.49	36.28
Britnico	51.70	32.43	32.90	35.26	35.26	31.49	32.43	31.49	31.49	31.49	31.49	34.31
Hyco	56.41	34.87	32.90	35.72	36.19	32.84	31.96	33.37	31.02	34.87	32.90	35.73
Nicofume	38.07	36.66	36.19	37.60	36.66	33.37	32.90	34.31	32.43	31.96	34.31	34.95

....

cc. to "Run Off" Point.

Nicotines	Soaps 0	1	2	3	4	5	6	7	8	9	10	Mean
Neotine	6.25	3.25	2.50	4.66	3.08	2.75	2.08	1.91	1.83	2.66	2.00	2.99
Britnico	6.87	2.37	2.17	3.25	3.08	2.25	2.41	2.17	1.83	2.25	2.33	2.81
Hyco	7.33	3.17	3.00	4.41	3.91	3.58	2.58	2.91	2.17	3.58	2.58	3.56
Nicofume	4.75	3.30	3.00	3.75	4.08	2.41	2.08	2.58	2.66	2.00	1.83	2.95

Table No. 47

Average Per Cent Kill of all Solutions Tested.

Soaps

	0	1	2	3	4	5	6	7	8	9	10
Neotine	37.5	75.4	90.7	69.4	74.3	76.5	91.3	75.6	90.9	77.0	51.7
Britnico	13.8	77.7	91.3	70.1	76.9	82.8	90.7	76.2	89.9	87.6	77.0
Hyco	18.1	32.3	78.0	38.5	55.2	56.1	64.0	28.3	63.2	28.3	70.5
Nicofume	76.9	88.5	91.4	83.8	79.9	88.1	89.3	87.8	92.9	88.0	90.2

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Table No. 47a

Adjuvant Values of Soaps.*

	1	2	3	4	5	6	7	8	9	10	Average
Neotine	37.9	53.2	31.9	36.8	39.0	53.8	38.1	53.4	39.5	51.7	43.5
Britnico	63.9	77.5	56.3	63.1	69.0	76.9	62.4	76.1	73.8	63.2	68.2
Hyco	14.2	59.9	20.4	37.1	38.0	45.9	10.2	45.1	10.2	52.4	33.3
Nicofume	11.6	14.5	6.9	3.0	11.2	12.4	10.9	16.0	11.1	13.3	11.1
Mean Adjuvant Value	31.9	51.3	28.9	35.0	39.3	47.2	30.4	47.6	33.6	45.1	
Mean Adjuvant Value for both strengths of each soap		41.1		31.9		43.2		39.0		39.3	

*Additional Per Cent of Kill Produced by Nicotine-Soap Solutions over Nicotines used without soap.

Table No. 48 Mean of Afjuvant Values and Physical Characteristics of the Soap Solutions for the four Nicotines tested.

	0	1	2	3	4	5	6	7	8	9	10
Adjuvant Values		31.9	51.3	28.9	35.0	39.3	47.2	30.4	47.6	33.6	45.1
pH Values	6.42	7.20	7.55	7.41	7.82	7.28	7.54	7.48	7.68	7.19	7.31
Surface Tension	51.58	34.80	33.96	36.78	36.31	32.88	32.43	32.78	32.08	32.92	31.96
cc. to Run Off Pt.	6.30	3.02	2.67	4.02	3.54	2.75	2.29	2.39	2.12	2.62	2.18

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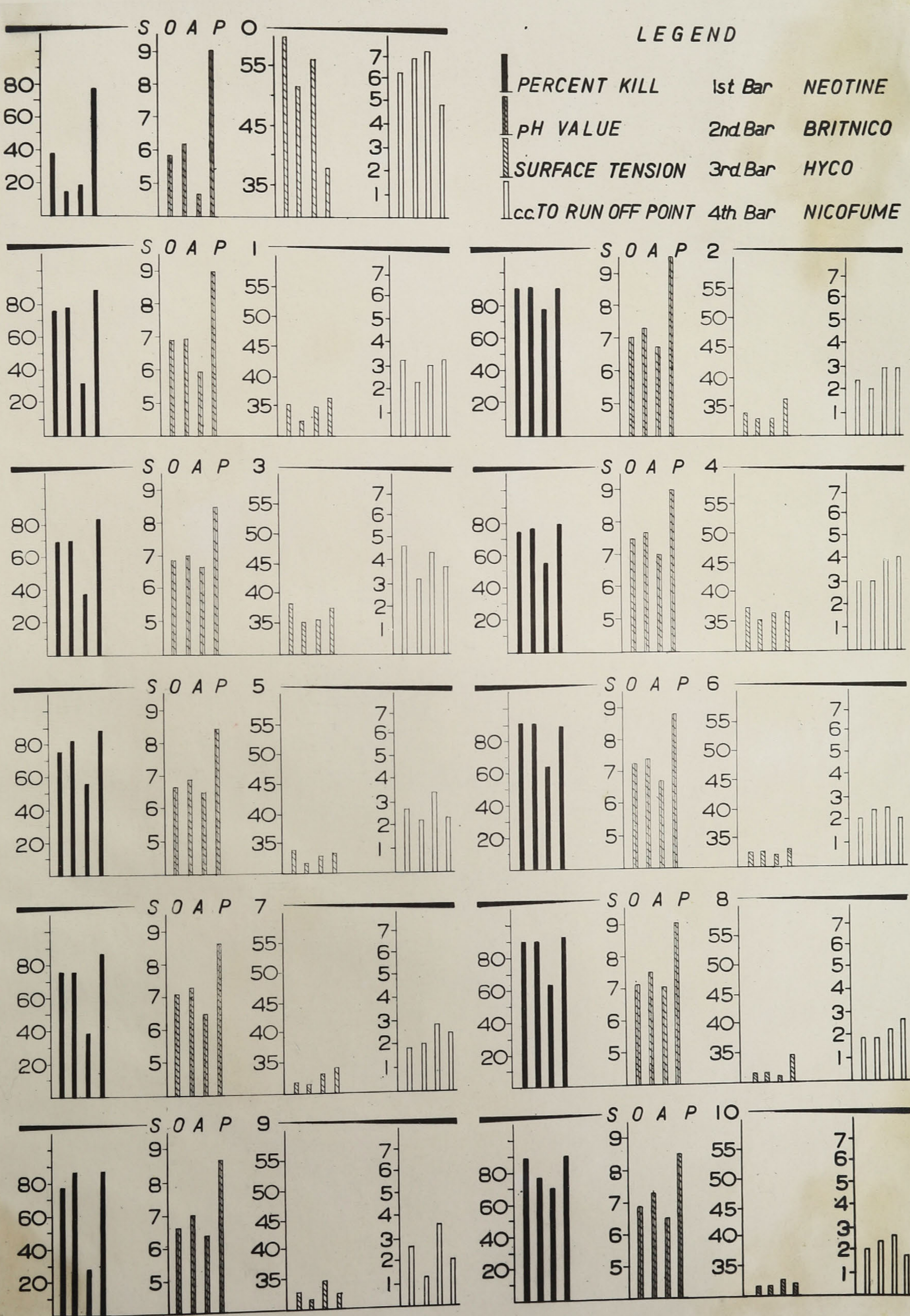
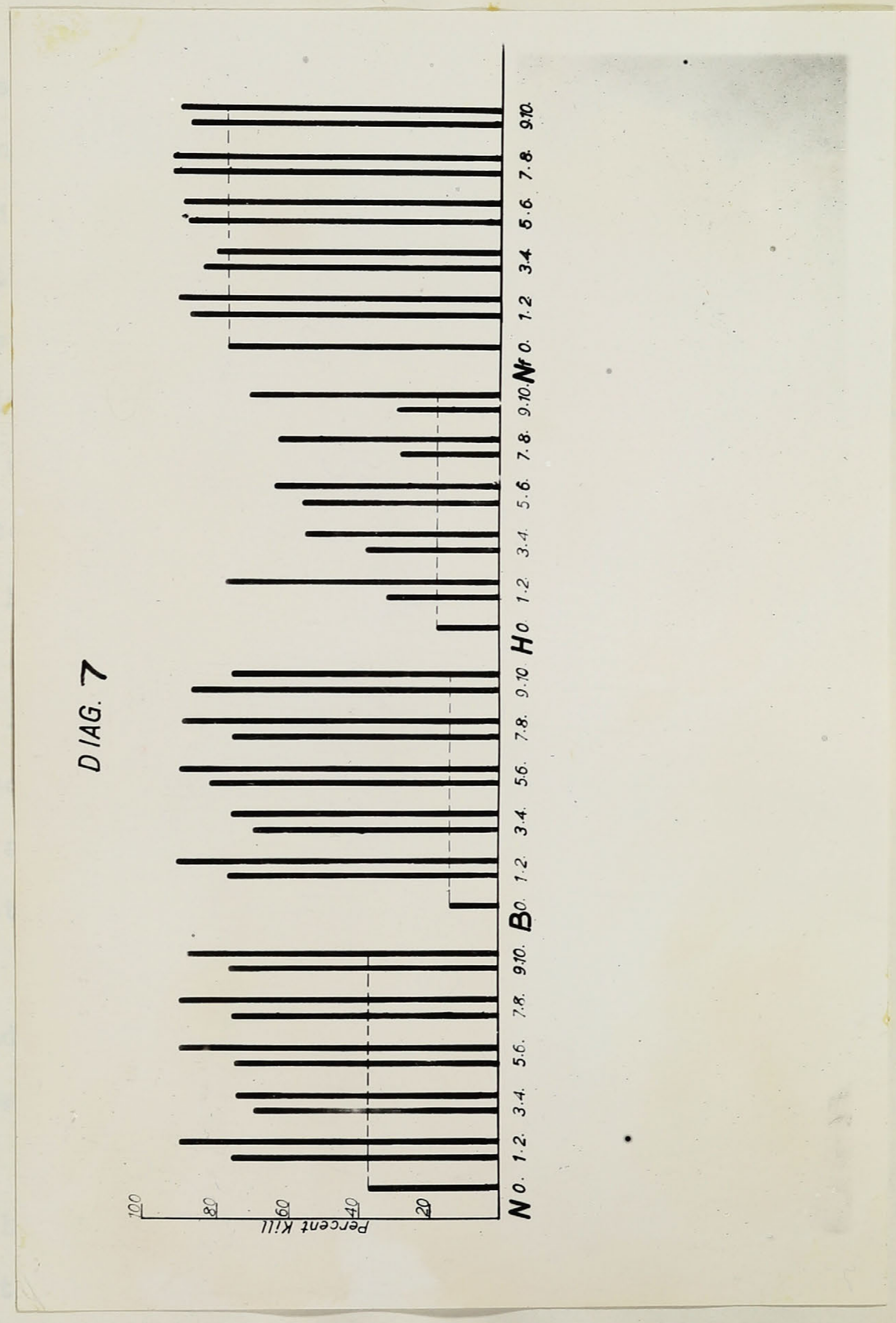
Mean of Adjuvant Values and Physical Characteristics of each Nicotine for the soaps tested.

	Adjuvant Values	pH Values	Surface Tension	cc. to Run Off Pt.
Neotine	43.5	6.91	36.28	2.99
Britnico	68.2	7.14	34.31	2.81
Hy co	33.3	6.44	35.73	3.56
Nicofume	11.1	8.92	34.95	2.95

DIAGRAM 6


LEGEND
PERCENT KILL
pH VALUE
SURFACE TENSION
c.c. TO RUN OFF POINT
1st Bar NEOTINE
2nd Bar BRITNICO
3rd Bar HYCO
4th Bar NICOFUME
SOAP 0
SOAP 1
SOAP 2
SOAP 3
SOAP 4
SOAP 5
SOAP 6
SOAP 7
SOAP 8
SOAP 9
SOAP 10


DIAG. 7
Percent Kill
100
80
60
40
20
N 0. 1.2. 3.4. 5.6. 7.8. 9.10.
B 0. 1.2. 3.4. 5.6. 7.8. 9.10
H 0. 1.2. 3.4. 5.6. 7.8. 9.10.
N 0. 1.2. 3.4. 5.6. 7.8. 9.10.

VIII. DISCUSSION OF RESULTS.

1. Comparison of Adjuvant Values of Nicotine Soap Solutions.

The average per cent of kill obtained by all solutions is shown in table 47 and illustrated by diagrams No. 6 and No. 7. In all cases the various brands of nicotines used without soaps have resulted in a lower per cent of kill than when used in combination with the soaps. Table 47a shows the additional per cent of kill of the nicotine-soap solutions over the per cent of kill resulting from the nicotines used without soaps. In two cases only, nicotine solutions in combination with .25 per cent strength of soap show a higher per cent of kill than the same nicotine in combination with .5 per cent strength of the same soap.

The high per cent of kill recorded in the Nicofume used without soaps is rather startling. It is obvious that the adjuvant values of the soaps in combination with this material cannot be as great as in the remaining nicotine sulphate series. In these series it is apparent that possibilities for greatest adjuvant values may be had in the Britnico series, the Hyco series coming second and the Neotine series coming last. Results of table 47a show that the soaps in combination with Britnico have produced the highest adjuvant values. In combination with Hyco, the soaps have produced the lowest adjuvant values of the nicotine sulphate series. In combination with Neotine, the soaps have resulted in adjuvant values between those received in the other two sulphate series. The low adjuvant values recorded in the Hyco series cannot be explained. The Neotine series, having an average kill of 37.5 per cent in the lot using no soap, have resulted in an average adjuvant value considerably higher than that recorded in the Hyco series. Hyco used without soap resulted in a kill of 18.1 per cent.

Results expressed in table 47a show that the .5 per cent sodium fish oil soap in combination with all nicotines used, gave the highest average adjuvant value. In considering both strengths of each soap used, the sodium oleate soaps resulted in the highest average adjuvant values. Had the .25 per cent strength of the sodium fish oil soap in combination with Hyco resulted in an adjuvant value equal to that recorded by the .25 per cent strength of the sodium oleate soap in combination with Hyco, the sodium fish oil soaps would have resulted in the highest average adjuvant values. The average adjuvant values of the potassium oleate and triethanolamine oleate soaps are approximately equal in the .25 per cent strengths and in the .5 per cent strengths.

The potassium fish oil soaps have resulted in the lowest average adjuvant values of the experiment. In each nicotine series the .5 per cent strengths of this soap has consistently given the lowest adjuvant values. The .25 per cent strengths of this soap have also given the lowest adjuvant values with the exception of the results recorded in the Hyco series.

2. Correlation of Toxicity and pH Values.

The pH values of the solutions tested are shown in table 46 and illustrated by diagram 6. The addition of soaps to the nicotine sulphates have increased the pH values of the solutions in all cases. It is also apparent that the solutions containing .5 per cent of soap have higher pH values than the solutions containing .25 per cent of soap. The pH values of the Nicofume series could not be exactly measured as the apparatus used was accurate only to a pH of 9. A correlation is shown between the per cent of kill obtained and the pH value of each solution containing soap in the three nicotine sulphate series. An increase in the per cent of kill is accompanied by an increase in the pH value.

The mean adjuvant and pH value of each soap used in combination with each nicotine is shown in table 48. It is apparent that the solutions containing the .5 per cent soaps have higher mean adjuvant and pH values than the solutions containing the .25 per cent soaps. In comparing the mean adjuvant and pH values of each nicotine sulphate series in table 48 a correlation between the two is noted. Hyco with the lowest mean adjuvant value has also the lowest mean pH value. Britnico with the highest mean adjuvant value has also the highest mean pH value. With the Nicofume series this correlation is not apparent, the adjuvant values being low because of the high per cent of kill obtained by the Nicofume used without soap. However, the high per cent of kill obtained may be correlated with the high pH values of this series.

3. Correlation of Toxicity with Surface Tension and cc's to Run off Point.

Surface tension determinations and cc's to run off point of all solutions are shown in Tables 46 and 48 and illustrated by diagram 6. The range of surface tension determinations of all soap solutions do not vary to any great extent. On the whole, the surface tension of the .5 per cent soap solutions are slightly lower than the .25 per cent soap solutions. In a few cases both strengths of soap show the same surface tension. Also in a few cases, the .5 per cent soap solutions show a higher surface tension than the .25 per cent soap solutions. There is considerable variation in the surface tension determinations of the nicotines used without soap. The low surface tension of the alkaloid is striking and is consistent with the high per cent of kill obtained by this solution. In the nicotine sulphate series, Britnico shows the lowest surface tension which is consistent with the per cent of kill obtained by these solutions. Inconsistency in the remaining two sulphate series is noted. Although the solutions with lower surface tensions have in general resulted in kills higher than the solutions with higher surface tensions, there is not sufficient evidence to say that surface tension can be directly correlated with toxicity.

In considering the cc's to run off point, table 46 shows some variation among the solutions tested. With the exception of four instances the .5 per cent soaps show lower cc's to run off points than the .25 per cent soaps. This is to be expected and is in correlation with the higher adjuvant values of the .5 per cent soaps. The mean cc's to run off point for each nicotine sulphate series is consistent with the mean adjuvant and ph values for these series. The Nicofume series does not show this correlation. The mean cc's to run off point for each soap in all series is not consistent with their adjuvant values. There is evidence of correlation between the cc's to run off point and the surface tension determinating. It is not established however, that the cc's to run off point are indication of the "toxicities" of the solutions tested.

IX. CONCLUSIONS.

1. An improved technique for the laboratory comparison of contact insecticides involving the use of *Drosophila melanogaster* as the test animal has been devised.

2. Various methods of rearing *Drosophila melanogaster* have shown that the adaptation of gum or candy jars as individual cultures for the production of flies is the most efficient of the methods tested.

3. Tests with various culture media have shown that a medium composed of partially peeled ripe bananas with liberal amounts of National Breweries Yeast upon a thin bed of fine sawdust is the most effective.

4. The presence of mould in the culture media was a serious detriment to the production of flies.

5. Results of feeding tests indicated flies lived longer in vials in which the feeding solutions were contained in both ends than in vials in which the feeding solutions were contained in one end only.

6. The natural mortality of flies sprayed with nicotine and soap solutions at strengths used was exceedingly low, as evidenced by the low mortality recorded in check plots.

7. In ten replicate sprayings of 0.5 per cent solutions of Neotine, Britnico, Hyco and Nicofume with samples of

flies taken from ten different gum jar cultures, inconsistency was established between replicate sprayings of flies from the same cultures and from different cultures. It is concluded that some unknown factor or factors such as age of flies at time of spraying, or humidity of cultures, affected the degree of toxicity.

8. It was shown that the susceptibility of flies to nicotine sprays was less for flies two days old than for flies three or four days old. Flies one day old were more susceptible to nicotine sprays than flies four days old.

9. It was shown that humidities of approximately 40, 75 and 100 per cent in which flies were aged until three days old did not affect the degree of toxicity.

10. It was shown that there was no significant difference in the susceptibility of flies to nicotine, when taken from aging cultures.

11. The toxicity of three commercial brands of nicotine sulphate and one brand of the alkaloid in combination with various soaps were compared. Analysis of variance showed the results to be consistent.

12. Results recorded showed that the alkaloid Nicofume, in combination with the various soaps gave the highest average per cent of kill. Of the three nicotine sulphates in combination with the various soaps, the Neotine and Britnico series gave approximately the same kill,

the Hyco series giving a much lower kill.

13. The mean adjuvant value for each brand of nicotine in combination with the various soaps varied considerably and may be expressed in descending order of mean adjuvant value as follows:- Britnico, Neotine, Hyco and Nicofume.

14. The adjuvant values of the soaps showed consistency in each brand of nicotine and for the strength of soap used.

15. The adjuvant values of the various soaps used, varied within a narrow range with the exception of the potassium fish oil soap, the mean adjuvant value of which was considerably lower than the others. Expressed in order of descending values the soaps place as follows:- sodium oleate, sodium fish oil, triethanolamine oleate, potassium oleate and potassium fish oil.

16. The mean pH values of the three brands of nicotine sulphate in combination with the soaps may be correlated with their mean adjuvant values. The alkaloid Nicofume, in combination with soaps showed the highest per cent of kill and also the highest pH value.

17. The mean pH values of the solutions containing the various soaps varied within a narrow range and cannot be directly correlated with their mean adjuvant values.

In considering each soap separately, both values are consistently higher in the solutions containing .5 per cent of soap than in the solutions containing .25 per cent of soap.

18. The mean surface tension of each brand of nicotine in combination with the various brands of soap did not vary to any great extent.

19. With the exception of potassium fish oil soap, the mean surface tensions of each soap did not vary to any extent. The mean surface tension of the potassium fish oil soap was higher than in the remaining soaps and the mean adjuvant value was correspondingly lower.

20. The mean "run-off-point" (in cc.) of the various nicotines in combination with the soaps cannot be directly correlated with toxicity or mean adjuvant value.

21. The mean "run-off-points" of the 0.25 and 0.5 per cent soaps in combination with various nicotines showed correlation with the mean adjuvant values of the soaps, the 0.5 per cent soaps in all cases showing lower run-off-points than the 0.25 per cent soaps.

22. It is significant to note the consistency displayed by the potassium fish oil soaps, having high mean run off points, high mean surface tensions and low mean adjuvant values, the two former characteristics being those which seem to indicate a poor adjuvant.

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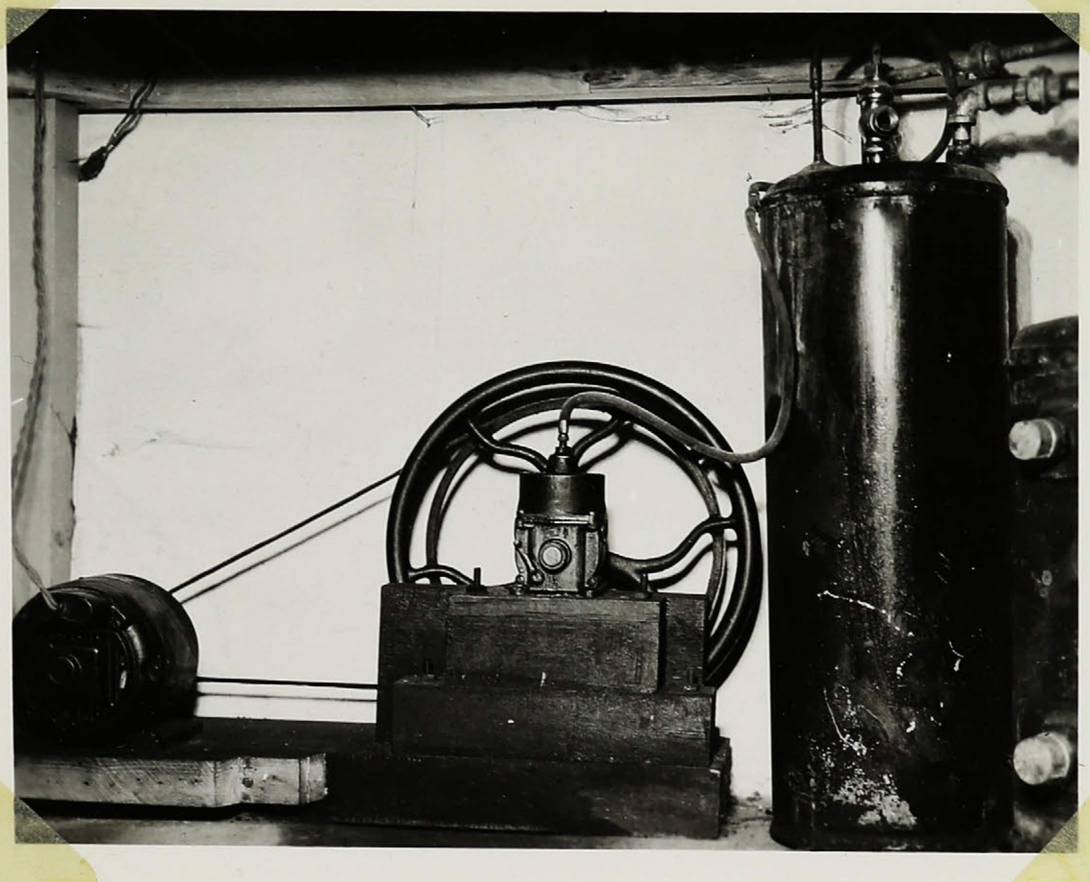

Fig. I

AIR PUMP AND MOTOR

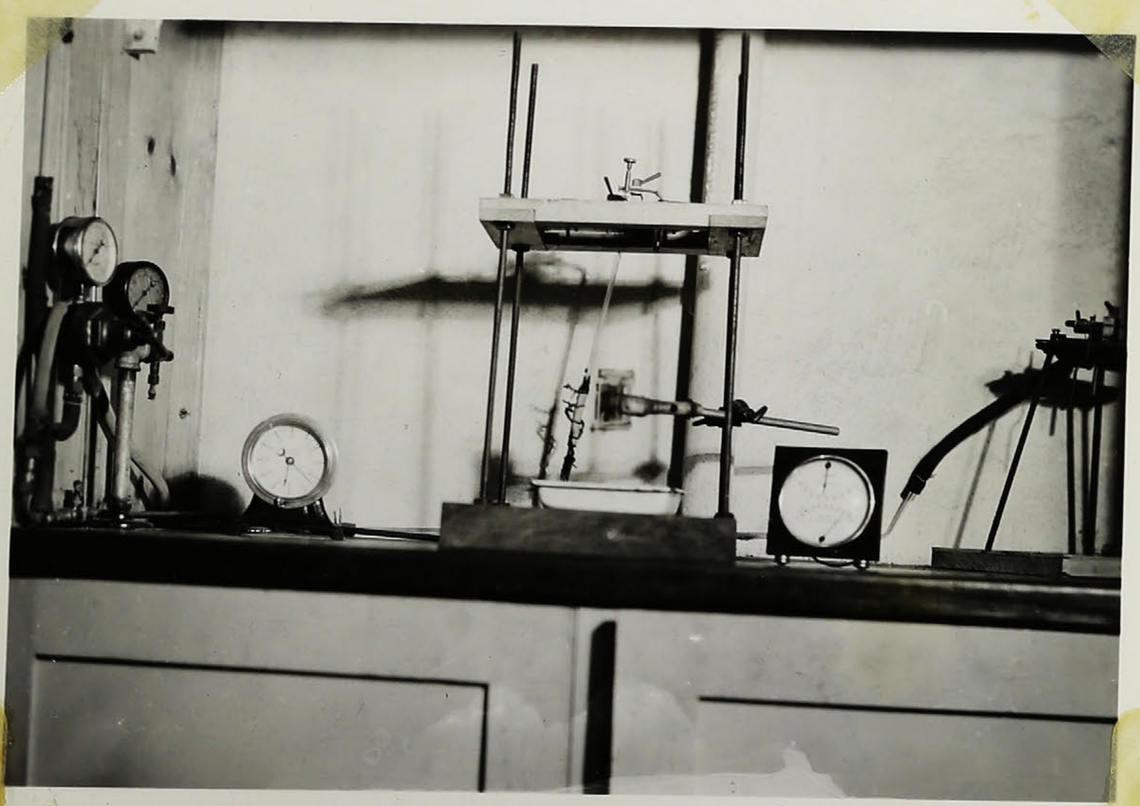

Fig. 2

PRESSURE GAGES AND ADJUSTABLE ATOMIZER SUPPORT

PLATE I

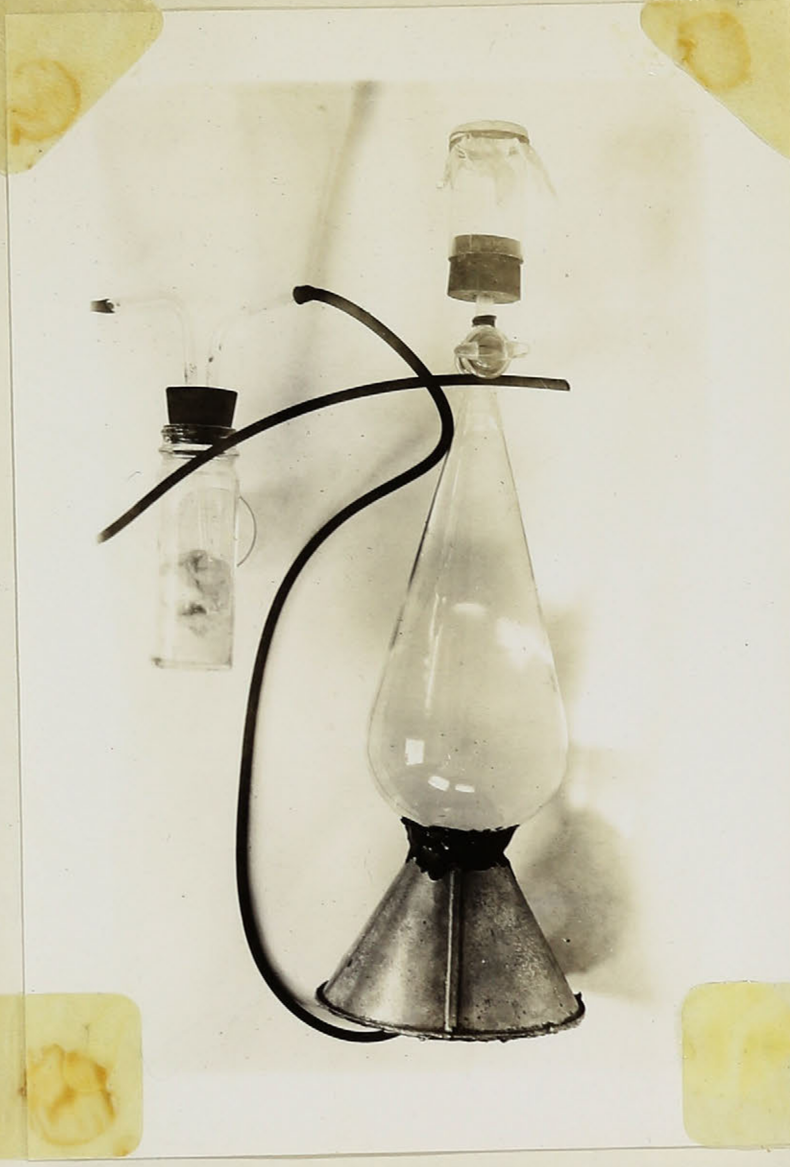

Fig. 3

APPARATUS FOR ADMITTING DROSOPHILA

TO SPRAYING VIALS. ($\frac{1}{4}$ natural size)

Fig. 4

FEEDING VIAL FOR SPRAYED FLIES

($\frac{3}{4}$ natural size)

PLATE II

Fig. 5

DROSOPHILA CULTURE IN GUM JAR

Fig. 6

AGING JAR FOR DROSOPHILA

PLATE III

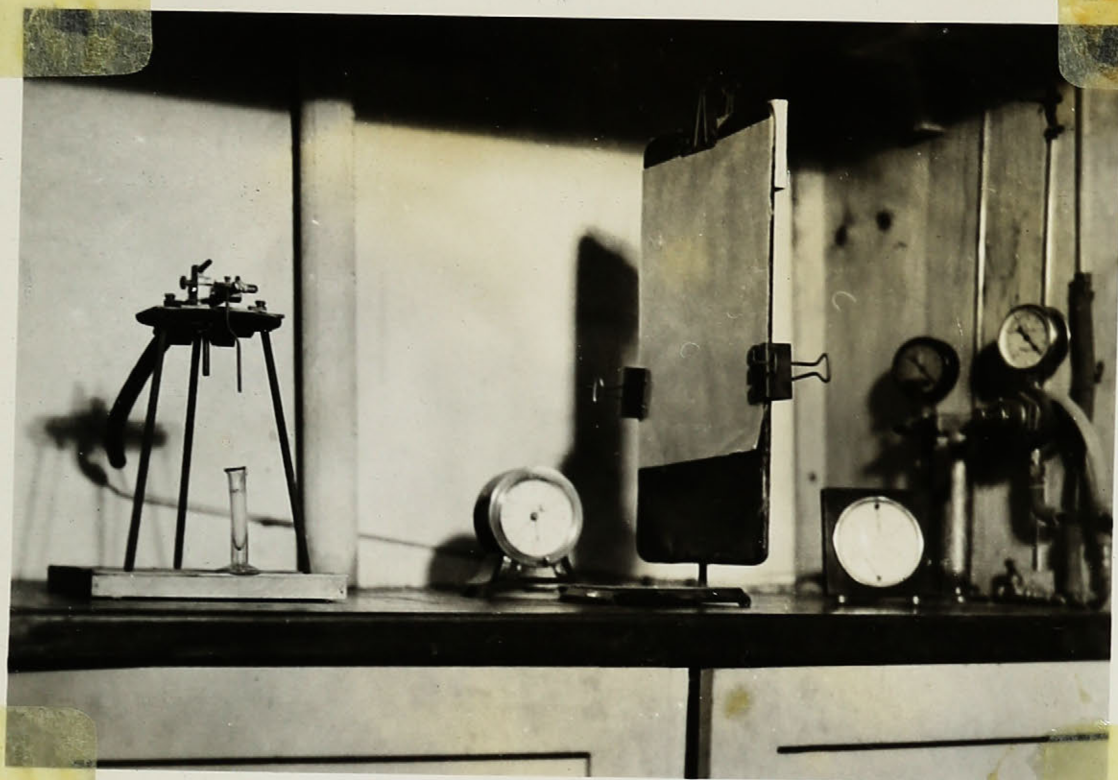

Fig. 7

APPARATUS FOR DETERMINATION OF RUN OFF POINT

Fig. 8

WIRE CAGE USED FOR AGING FLIES

FOR USE IN HUMIDITY TESTS

PLATE IV